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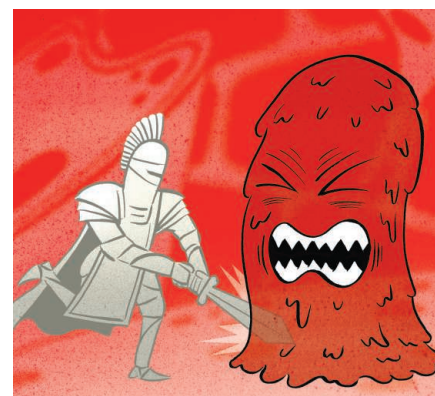
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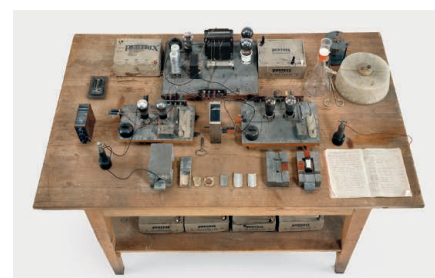
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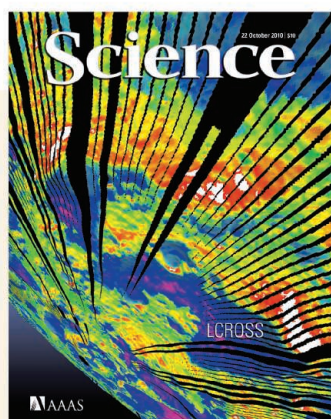
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Surface temperature map of the south polar region of the Moon acquired by the Lunar Reconnaissance Orbiter Diviner Lunar Radiometer Experiment. Blue and violet areas are intensely cold impact craters. The Lunar Crater Observation and Sensing Satellite (LCROSS) spacecraft struck one of these craters (near the center of the frame), revealing the presence of water ice and other frozen volatiles. See the six research papers beginning on page 463.

Image: David A. Paige/UCLA/JPL/GSFC/NASA

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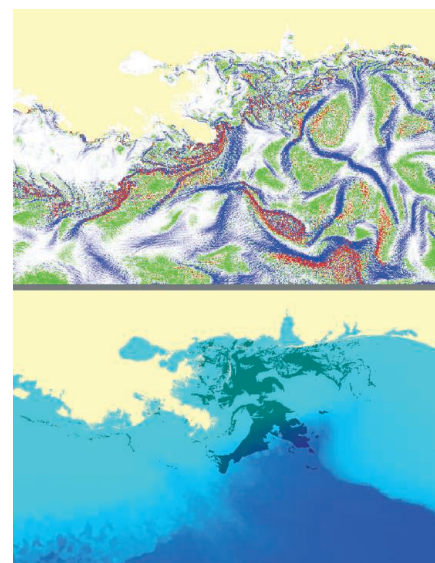
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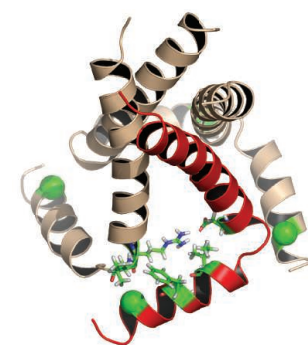
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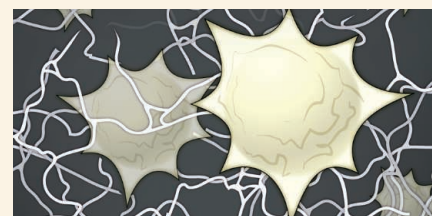
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ADVANCING SCIENCE, SERVING SOCIETY



Bruce Alberts is Editor-in-Chief of *Science*.

An Education That Inspires

WHY IS IT THAT CHILDREN, WHO ENTER SCHOOL AT AGE 5 FILLED WITH EXCITEMENT AND WONDER about the world, often become bored with education before their teenage years? How might the United States produce a more engaging education system, one that allows a child with a specific fascination to explore that interest in depth as an integral part of his or her early education? Here I sketch a possible plan based on science, technology, engineering, and math (STEM) awards that would be largely earned through student activities outside of school.

The idea has been partly inspired by the U.S. Advanced Placement (AP) system of courses and exams, which makes a first-year college-level education in selected subjects available to high school students. As a nationally recognized standard of achievement, passing an AP course is a mark of success for both students and schools. High schools now strive to increase the number of students taking such courses, and this nongovernmental but nationally certified program has been rapidly growing in popularity. Could a nationally validated set of “STEM challenge awards,” designed for students at earlier stages of schooling, similarly motivate schools and school systems to value a new type of achievement?

I suggest that the proposed STEM challenge awards be modeled on the achievement badges that youth organizations around the world have developed to promote the active learning of specific subjects in depth. For example, the Boy Scouts of America allows more than 100 different merit badges to be earned, each focused on a specific topic such as Plant Science or Lifesaving.* In addition to this large selection, each badge provides a young person with a variety of options. Thus, to earn a Plant Science merit badge, a scout can choose between agronomy, horticulture, or field botany. Most learning experiences are active ones, such as “Select a study site that is at least 100 by 100 feet. Make a list of the plants in the study site by groups of plants: canopy trees, small trees, shrubs, herbaceous wildflowers and grasses, vines, ferns, mosses, algae, fungi, lichens. Find out which of these are native plants and which are exotic (or non-native).” This is infinitely more interesting than a typical school experience in which students memorize the names of plants and their parts from pictures in a textbook, often without encountering the actual object.

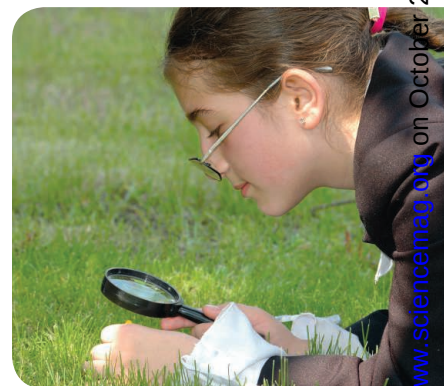
A STEM challenge award program might provide 100 different challenges to choose from at each level of schooling (for example, sets of awards of increasing difficulty for ages 5 to 8, 9 to 13, and 14 to 18), on subjects ranging from reptiles to Web design. Scientific and engineering societies in each discipline could create the requirements for many awards, as could industry groups or government agencies such as the U.S. National Aeronautics and Space Agency. But a single umbrella organization would be needed to certify the contents of the award projects, as well as the mechanisms used to judge and record their completion. Such national certification would be critical for the awards to have a substantial positive impact, serving as a widely recognized, valid mark of success for both students and school districts.

The most ambitious and revolutionary part of this plan supplements the teachers in schools with adult volunteers, each serving as an expert for a particular STEM challenge award. To earn a merit badge, a scout must demonstrate to a qualified adult volunteer (a “counselor” for that badge) that he has satisfied that badge’s requirements. In a similar way, many thousands of adults with science and technology backgrounds would be enlisted as counselors, both to help teachers and to judge each student’s performance, making full use of modern communications tools. A great many scientists and engineers would be willing to contribute to improving science in schools if an efficient and effective way for them to do so could be generated. And their contributions could truly inspire today’s students.

— Bruce Alberts

10.1126/science.1199138

*www.scouting.org/scoutsource/BoyScouts/AdvancementandAwards/MeritBadges.aspx.



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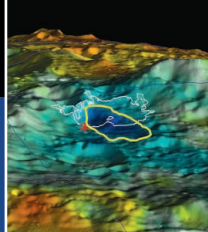
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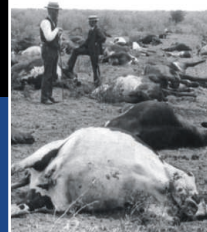
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ENVIRONMENT

After Red Mud Flood, Scientists Try to Halt Wave of Fear and Rumors

KOLONTÁR, HUNGARY—Shortly after a red river from hell tore through this village on 4 October, destroying lives and homes, Hungary's junior minister for environmental affairs, Zoltán Illés, said aloud what many feared: Nobody really knew what was in the caustic sludge—a byproduct of the aluminum industry whose pH can reach a dizzying 13—that had broken through a nearby reservoir.

But scientists at the Institute of Materials and Environmental Chemistry in Budapest begged to differ. The institute, part of the Hungarian Academy of Sciences, has done research on the toxic residue—which chemists call “red mud”—for decades. “We know red mud better than we know our wives,” says László Kótai, a chemical engineer at the institute.

Kótai is part of a nine-member scientific task force, called together by the Hungarian government, which has found itself battling not just mud but also a rising tide of rumors and misinformation. Illés, for instance, said that the sludge was radioactive and could cause cancer, and the environmental group Greenpeace announced that it contained dangerous levels of heavy metals. Those claims led to fears of a widespread ecological catastrophe—including a poisoning of the Danube, one of Eastern Europe's major rivers. But Illés has dropped his claim about radioactivity, and scientists say Greenpeace's report was alarmist and unscientific.

Two weeks after the disaster, Kótai and other scientists say its environmental impact may be less severe than widely broadcast images suggested at first. They don't want to diminish the human tragedy: Nine people have died and more than 100 have been injured; many suffered burnlike wounds from the sludge's alkalinity. But much of the sludge can be removed physically and taken back to one of the company's intact reservoirs; the remainder can be neutralized with mild acids, says János Szépvölgyi, an environmental chemist who heads the task force. Task force members predict only minimal effects on the

ecology of the Danube and don't think drinking water is at risk. “It's not as dramatic as it seems,” says Tamás Németh, the academy's general secretary and a soil scientist himself. “We can handle this.”

About a million tonnes of red mud poured out when the dam on reservoir 10 of the Ajkai



Red menace. László Kótai, a chemical engineer, talks to police officers in Devcester.



Timföldgyár alumina factory near Kolontár broke just after noon on 4 October. The sludge is formed during the so-called Bayer process, when bauxite ore is pressure-cooked with sodium hydroxide in giant reactors to extract aluminum hydroxide. The residue was stored in giant reservoirs right next to the factory, as it is at similar facilities around the world.

What caused the break is still under investigation. Some environmental groups had suggested that Hungary's red mud reservoirs, a legacy of the communist era, were poorly monitored and prone to breaks. The Hungarian task force, which also includes biologists and ecologists, has set out to map the impact of the release and provide the government with advice on how to limit the harm. Aside from the sheer physical impact of the initial wave—some Kolontár residents likened it to a red tsunami—the sludge's main problem is its deadly alkalinity, says Szépvölgyi. The Torma, a small stream near the village that ultimately flows into the Danube, turned red and is now

almost completely lifeless. But the environmental damage downstream was reduced, says Szépvölgyi, partly by dilution and possibly because government workers dropped large amounts of fertilizers and calcium sulfate into the rivers to bring down the pH. Elevated pH values of between 8 and 9 were measured locally in the Danube for a few days, but they have come down, says Szépvölgyi. No major fish die-offs in the Danube have been reported.

Nor do task force members believe heavy metals will be a major problem. During a press conference on 8 October, Greenpeace

announced that red mud from Kolontár contained high levels of arsenic, chromium, and mercury, suggesting that illegal dumping had occurred in the sludge pond before it broke. For arsenic, the level was 25 times higher than the limit for drinking water, the group said.

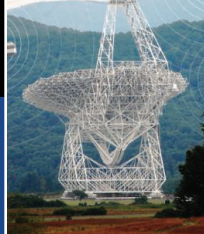
But that's a nonsensical comparison because nobody is planning on drinking the sludge, says Szépvölgyi. A better reference is the arsenic level allowed in organic sludges that Hungarian farmers are permitted to spread on their lands; the level measured by Greenpeace was just slightly above that. Szépvölgyi also criticizes the group for basing its findings on a single sample. The sludge wasn't homogenous, he says, and the task force's own measurements have found varying levels of heavy metals at different locations—but none dangerously high, Szépvölgyi asserts.

Greenpeace spokesperson Szabina Moses says the group stands by its findings and that the academy scientists are downplaying the risks. “We're talking about arsenic. I don't



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have to be a scientist to feel that that is dangerous," Moses says.

Initial news stories suggested that Kolontár and Devecser, a bigger town farther downstream, might have to be abandoned altogether. That was an exaggeration, task force members say. When Kótai gave *Science* a tour of the area last week—expertly talking his way past police roadblocks—cleanup activities in Kolontár were in full swing. Trucks were hauling away tonnes of red mud; a few homes were clean again, and a handful of residents, looking somber, were returning. Many yards and sidewalks were covered with calcium sulfate. The compound—which makes streets look as if they're covered with dirty snow—not only helps neutralize the mud but also prevents it

from becoming an easily dispersible powder once it dries. Instead, the mix forms a cake that can be removed with shovels.

What will happen to the surrounding farmland? The layers of red mud there vary from 1 or 2 centimeters to more than 10 cm thick. Some will need to be removed, but small quantities can probably be mixed in with the soil, says Németh. The alkalinity can be neutralized with mild acids, such as acetic acid or humic acid, a complex organic mixture that occurs naturally in the soil. Studies will have to show whether crops can be grown safely in these areas, Németh adds, but it might be wise to stick with biofuel crops or trees for the foreseeable future. "I don't think anybody will want carrots and potatoes from this area anyway."

As *Science* went to press, another breach from the same reservoir threatened. But a new dam would divert most of the sludge, even if that happened, says Németh. What's more, the reservoir contains far less fluid, so any escaping mud would travel slowly and only a couple of hundred meters, models show.

Amid the media frenzy, scientists say they are trying to be the voice of reason. When Kótai encountered one roadblock in Devecser, two police agents wearing rubber boots and facemasks asked if they were at risk as they guarded a street full of deserted homes. Kótai tried to reassure them. "They are scared," he said after he got back into his mud-covered Volvo. "They have heard so many stories."

—MARTIN ENSERINK

EXOPLANETARY SCIENCE

First Goldilocks Exoplanet May Not Exist

Astronomers have begun debating whether the first claimed habitable planet circling another star is a figment of their data analysis. At a meeting last week in Turin, Italy, a group of exoplanet hunters announced that its observations show no sign of Gliese 581g, the Goldilocks planet on which conditions are just right for harboring life. Only a couple of weeks earlier, a rival group had announced with much fanfare its discovery of the long-sought Earth-like, habitable planet.

"Of course, it's not easy to definitively rule out something," says exoplanet astronomer Ray Jayawardhana of the University of Toronto in Canada, but the "results presented here at Turin are at least raising some doubts."

Such controversy is not surprising. To be detectable, Gliese 581g (if it's there) must gravitationally tug on its star strongly enough for astronomers to recognize the resulting stellar wobble in observations of the varying frequency of starlight. But at about three times the mass of Earth, Gliese 581g would be among the smallest of known exoplanets, and four other planets—three of them much more massive—are tugging on the same star.

To sift out any particularly subtle stellar wiggles, exoplanet hunter Steven Vogt of the University of California, Santa Cruz, and colleagues combined

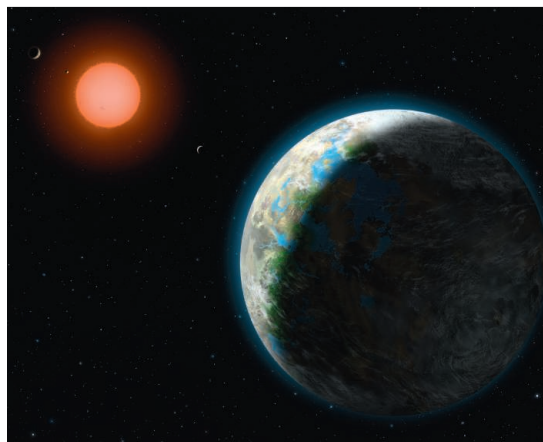
two series of observations. One 11-year-long data set consisted of 122 measurements made by Vogt and colleagues. The other set was 4.3 years long and consisted of 119 measurements published earlier by a rival consortium headquartered at the Observatory of Geneva in Switzerland. In a 29 September press conference and in a paper subsequently published in *The Astrophysical Journal*, the American group announced the discovery of an exoplanet massive enough to hold on to a life-sustaining atmosphere, orbiting its star every 37 days. That close to a small, faint star, a planet's water could remain liquid.

The first known habitable planet looked

like a done deal until a member of the Swiss group, exoplanet hunter Francesco Pepe of the University of Geneva, made his announcement at the Astrophysics of Planetary Systems meeting in Turin. Since publishing the data set the American group had used, the Swiss had extended their record to 6.5 years, including a total of 180 measurements. "We do not see any evidence for a fifth planet ... as announced by Vogt *et al.*," Pepe wrote to *Science* in an e-mail from the meeting. On the other hand, he added, "we can't prove there is no fifth planet."

Confirming or denying Gliese 581g will likely require agreement that someone's data analysis went awry. If data quality turns out to be a wash, the two groups' different approaches to data analysis may offer an explanation, says astrophysicist Alan Boss of the Carnegie Institution for Science in Washington, D.C., who was at the Turin meeting. The Swiss group's analysis assumes the orbits of Gliese 581's planets to be elongated in accord with the observations, he notes. If those orbits are in fact circular, as the American group believes, the supposed elongation could mask the presence of a smaller fifth planet. But even if data-analysis issues are not ironed out, all agree, a few more years of observation should do the trick.

—RICHARD A. KERR



Fuzzy concept. Is the first habitable exoplanet imaginary?

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PLANETARY SCIENCE

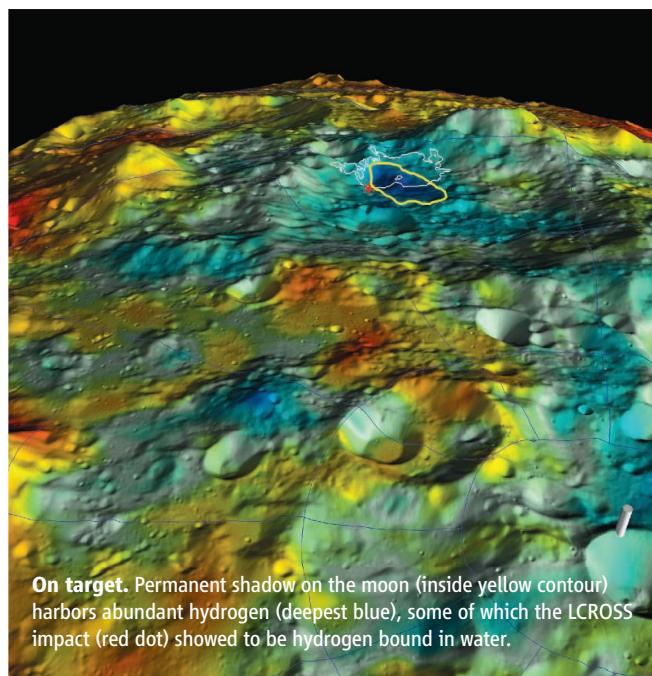
How Wet the Moon? Just Damp Enough to Be Interesting

"The Moon Is Wet," read many a headline in the past year, at times with an exclamation point. That's an overstatement, with or without the exclamation point, says lunar scientist Paul Spudis of the Lunar and Planetary Institute in Houston, Texas. But "the old statement that the moon is 'bone-dry' is not correct either. What we have found is that the moon has a hydrosphere, it has a water cycle."

But the moon's hydrosphere is nothing like Earth's watery regime. None of the moon's water is ever liquid. Water in its reservoirs can be imperceptibly sparse, flows into its reservoirs may proceed a few molecules at a time, and none may ever leave. And, as seen in the five reports of new water-related results from the moon beginning on page 463, many enigmas remain.

A year ago, researchers found the first water on the moon, frozen water buried in Cabeus crater near the south pole. Blasted into view by a plunging spent rocket stage (*Science*, 19 March, p. 1448), this subsurface ice is now pegged at only about 6% by mass of the top meter or two of lunar soil, according to LCROSS (Lunar Crater Observation and Sensing Satellite) mission team members reporting on page 463. This LCROSS water was the prize in a decades-long search driven most recently by NASA's push to sustain astronauts returning to the moon. But since the LCROSS mission, the Obama Administration and now Congress have turned away from sending humans back to the moon (*Science*, 1 January, p. 18).

There's still plenty in recent results to intrigue researchers, however. For starters, the controversial radar probing of the subsurface that ignited the hunt for lunar water (*Science*, 13 March 1998, p. 1628) was misinterpreted, the LCROSS result shows. No radar could detect such sparse ice. And the remote probing for subsurface hydrogen that led LCROSS team members to Cabeus was misleading. The Lunar Exploration Neutron Detector (LEND) instrument flying on NASA's Lunar Reconnaissance Orbiter (LRO) (p. 483) picked up a strong hydrogen signal. But it wasn't mostly from water's hydrogen, as team members originally thought. LRO's Lyman Alpha Mapping Proj-



On target. Permanent shadow on the moon (inside yellow contour) harbors abundant hydrogen (deepest blue), some of which the LCROSS impact (red dot) showed to be hydrogen bound in water.

ect found that at least as much hydrogen in the LCROSS impact plume took the form of molecular hydrogen, H_2 , as was locked up in H_2O (p. 472). How the molecular hydrogen got there remains unclear.

Results from LCROSS, LRO, and other recent moon missions confound as well as illuminate. Radar probing from India's recently deceased Chandrayaan-1 orbiter picked up no reflections from LCROSS's Cabeus crater, consistent with sparse ice. But strong reflections from a couple of dozen craters around the lunar north pole suggest an icy bonanza: massive, nearly pure ice just beneath the crater floors. "How that would come about I haven't a clue," says Spudis, who is principal investigator of the Chandrayaan-1 radar.

The conventional explanation for ice in polar craters like Cabeus—whose floor is in permanent shadow and thus hovers near 40 degrees above absolute zero—is that icy asteroids or comets strike somewhere on the moon, and some of the resulting water vapor reaches a permanently shadowed crater's deep chill and freezes out there. But such cold trapping would only fill the empty space in the soil, not form nearly pure ice. No one really knows how nearly pure subsurface ice would form.

Adding further confusion, LEND team members also report that surprisingly high amounts of hydrogen lurk near the south pole in places that get at least some sunshine. It

turns out that temperatures measured from LRO and reported on page 479 can be low enough to allow billion-year-old ice in sunlit areas as long as there are at least a few centimeters of overlying, insulating soil. More perplexing is LEND's failure to find abundant hydrogen in many permanently shadowed craters that are just as cold as Cabeus. "The data's not pointing to a single process for emplacing water," concludes Anthony Colaprete, LCROSS's principal investigator from NASA's Ames Research Center in Mountain View, California.

Even at its moistest, the lunar hydrosphere isn't very wet. Several teams of spectroscopists observing the moon from different space platforms have reported detecting water on the surface (<http://scim.ag/whiff-of-water>). But they are talking about layers just a few molecules thick.

One team reported a decrease in such surface water as the lunar day wore on, suggesting that heating frees water stuck to the surface to fly about the vanishingly thin lunar "atmosphere"—another water reservoir—until again hitting and sticking to the surface. This leapfrogging process might explain how water from impacting meteorites can bounce into a cold trap instead of escaping from the moon entirely.

Reports of wetness in rare minerals that Apollo astronauts brought home might also be overblown. From those analyses, some geochemists have inferred that the ancient moon's interior harbored a few tens of parts per million of water. But since those claims, a group analyzing chlorine isotopes in Apollo samples concluded that the lunar interior has always been bone-dry at less than 10 parts per billion (<http://scim.ag/beneath-the-surface-of-the-moon>). At stake, hydrologically speaking, is whether gas-emitting volcanoes on the moon ever contributed to the lunar hydrosphere.

As Colaprete notes, the question of lunar water "is complicated." Planetary scientist David Paige of the University of California, Los Angeles, thinks "we've done what we can remotely. My guess is the ultimate solution is in situ exploration." If the humans aren't game, bring on the robots.

—RICHARD A. KERR

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ANIMAL SCIENCE

Rinderpest, Deadly for Cattle, Joins Smallpox as a Vanquished Disease

Rinderpest, an infectious disease that has decimated cattle and devastated their keepers for millennia, is gone. The United Nations Food and Agriculture Organization (FAO) announced on 14 October in Rome that a 16-year eradication effort has succeeded and fieldwork has ended. "This is the first time that an animal disease is being eradicated in the world and the second disease in human history after smallpox," FAO Director-General Jacques Diouf said in his World Food Day address in Rome the next day.

"It is probably the most remarkable achievement in the history of veterinary science," says Peter Roeder, a British veterinarian involved with FAO's Global Rinderpest Eradication Programme (GREP) from its launch in 1994 until he retired in 2007. For the veterinarians who participated in the effort, the achievement is particularly poignant. "I thought this could not be done before I passed away," says 79-year-old Yoshihiro Ozawa, who, as FAO's chief veterinary officer in the late 1980s and early 1990s, helped launch GREP. "I'm very lucky to see the end of [rinderpest]."

One formality remains: The Paris-based World Organisation for Animal Health (OIE) still must complete the certification of a handful of countries as rinderpest-free. OIE is likely to adopt an official declaration recognizing the demise of the disease at its May assembly. Meanwhile, animal-disease fighters have already been applying lessons learned from the rinderpest campaign and pondering which animal disease might be the next target for eradication.

Although nearly forgotten in much of the West, as recently as the early 1900s, outbreaks of rinderpest—from the German for "cattle plague"—regularly ravaged cattle herds across Eurasia, often claiming one-third of the calves in any herd. The virus, a relative of those that cause canine distemper and human measles, spreads through exhaled droplets and feces of sick animals, causing fever, diarrhea, dehydration, and death in a matter of days. It primarily affects young animals; those that survive an infection are immune for life.

When the virus hit previously unexposed herds, the impact was horrific. In less than a decade after the virus was inadvertently introduced to the horn of Africa in 1889, it spread throughout sub-Saharan Africa, killing 90% of the cattle and a large proportion of domestic oxen used for plowing and decimating wild buffalo, giraffe, and wildebeest populations. With herding, farming, and hunting devastated, famine claimed an estimated one-third of the population of Ethiopia and two-thirds of the Maasai people of Kenya and Tanzania.

European countries gradually eliminated rinderpest in the early 20th century through conducting disease surveillance, controlling animal shipments, and culling sick and exposed animals. It continued to afflict Asia and Africa in the second half of the century,



Lethal legacy. Rinderpest killed 90% of domestic cattle in sub-Saharan Africa in the late 1800s, including these from a South African herd in 1897.

re-emerging after at least one eradication campaign was ended in the mistaken belief that the virus had been conquered.

In 1994, when rinderpest was entrenched in central Africa, the Arabian Peninsula, and a swath stretching from Turkey through India and to Sri Lanka, FAO brought together three regional rinderpest-control programs into GREP and set the goal of eliminating the disease by 2010. "At times, I was pessimistic that we were going to get anywhere," says Roeder. The key technical breakthrough was the recognition that the virus was re-emerging from just a handful of reservoirs that could be the targets of intensive surveillance and vaccination campaigns. In 1990, Jeffrey Mariner, then at Tufts University School of Veterinary

Medicine, had developed an improved vaccine that did not require refrigeration up to the point of use. This allowed vets and technicians to backpack vaccine into remote areas. One of the reservoirs was in the heart of war-torn eastern Africa, where vet services had broken down and international agencies dared not send personnel. GREP relied on local pastoralists to track the disease and on trained community animal-health workers to administer the vaccine to quell outbreaks.

Roeder says a second key to success was that as GREP made progress, countries initially skeptical or reluctant to share information on their rinderpest problems started cooperating. The virus was last detected in 2001 in wild buffaloes in Meru National Park in Kenya, on the edge of the Somali ecosystem.

What comes next? Some veterinary experts question whether the international community is ready to take on another massive eradication campaign, but one disease mentioned as a possible eradication target is peste des petites ruminants (PPR), which is highly contagious and lethal among sheep and goats. Related to the rinderpest virus, the PPR virus has long circulated in central Africa, the Middle East, and the Indian subcontinent and has recently spread to Morocco. Jan Slingenbergh, an FAO infectious diseases specialist, is cautious. PPR isn't as devastating as rinderpest once was, he says, and available vaccines are not as effective as the rinderpest vaccines, so disrupting viral transmission would be even harder. Slingenbergh says

FAO will attempt progressive control of PPR, starting in priority areas and expanding as studies indicate the best way forward. But the focus will be on "a package of small ruminant health care rather than singling out one disease," he says.

One of the final steps of the rinderpest-eradication program is tracing virus samples held in labs around the world and then sequestering them in a small number of secure international depositories, says Roeder. The GREP secretariat will continue to watch for the disease, he says, noting that he personally monitors disease outbreak Web sites and international news for any hint of a rinderpest revival. Rinderpest might be gone, but it's not forgotten.

—DENNIS NORMILE

CHINA CENSUS

1.3 Billion Divided by 6.5 Million, And Watch That Floating Decimal

Taking an accurate tally in the world's most populous and mobile country is already a formidable task. But when 6.5 million census takers fan out across China on 1 November, they will face an additional challenge: getting a handle on a swelling migrant, or "floating," population. The Chinese government has put "a lot of attention on enumerating the floating population as accurately as possible," says Duan Chengrong, a demographer at Beijing's Renmin University who helped design the sixth national census. China's unique solution this year—adding presumed residents to an actual head count—unsettles some demographers.

In the 2000 census, enumerators iden-

is not alone.

This year, to reduce undercounting, census takers will bolster registration records with a partial head count, using digital maps to pinpoint homes in far-flung or unlikely areas. "With geographic information systems, we can see clearly which houses are in which census districts," says Li Xiru, who is overseeing the technology's introduction for the National Bureau of Statistics, which administers the census. The 6-month residency rule still applies for floaters, Li says, but enumerators will try to tally recent migrants in their villages by asking families about absent members. Foreigners and residents of Hong Kong and Macao will be counted for the first time.

An approach that combines registration data with a head count and detailed interviews is "unique in the world," says Zhongdong Ma, a demographer at Hong Kong University of Science and Technology. The compromise acknowledges that household registration information is increasingly irrelevant. In recent decades, over 100 million rural residents have flooded China's cities in search of work. Many middle-class Chinese, meanwhile, have fled to the suburbs while maintaining apartments and residence permits in the city.

The novelty of this year's census worries observers like Qiao Xiaochun, a demographer at Peking University's Institute of Population Research. After working as a census officer in 1982, he published a book proposing that China switch to a de facto count, in which everyone is tallied in the districts where they are found on

census day, regardless of registration. This year's change is a step in the right direction, he says, but it opens alarming loopholes. People "may be enumerated twice," Qiao says. "Or they may not be enumerated at all."

Accuracy will depend on the enumerators, mostly short-term workers recruited in the past few months who will disperse throughout China over 10 days. They will help respondents fill out a form listing 15 questions—more than ever before. A randomly selected 10% of Chinese will com-

plete a long form that asks about things like education, housing, and marital status. The government is "going to great lengths to make a really detailed record," says Kam Wing Chan, a geographer at the University of Washington, Seattle, who studies migration in China. But that thoroughness, he says, has drawbacks: More questions could reduce the response rate.

In 1982, when China introduced modern census-taking techniques, many Chinese lived in closely monitored work units. Today people are busier, more mobile, and more private. Everyone from unmarried couples worried about disapproving neighbors to tax-evading tycoons may have reason to dread the enumerators.

In recent months, a barrage of propaganda has assured wary Chinese that information will be used only for calculating population data—and reminded them that the census is essential for planning schools, nursing homes, and transportation networks. But some local authorities implementing the count have already reneged on the promise to safeguard individual rights. During a census rehearsal in Beijing last August, plainclothes police posing as enumerators detained dissident writer Xie Chaoping.

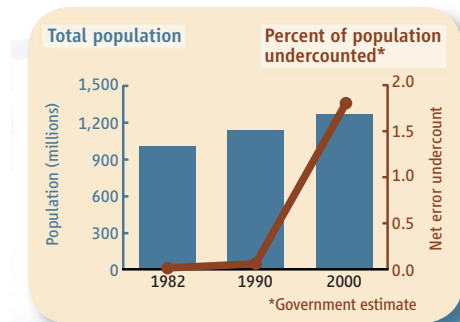
Other problems are disappearing with economic development. In 2000, some couples who had babies outside the one-child policy did not report those births, and as many as a quarter of young children went uncounted. Surveys suggest that the number of second and third births has fallen since then, so today's parents have fewer children to hide.

Along with the total population, scholars look forward to an updated sex ratio at birth—a 2005 sample survey found that 120.5 boys were born for every 100 girls, the most skewed ratio in the world—along with new fertility and mortality rates. The U.S. Census Bureau will use those numbers to revise its estimate for when China's population will peak, says Daniel Goodkind, a demographer at the bureau. The current forecast is 2026.

Beyond those broader tabulations, demographers wonder how much they'll learn. The Chinese government does not publish detailed data that allows demographers to make sense of its census tweaks. "How the census is adjusted is not known," says Cai Yong, a demographer at the University of North Carolina, Chapel Hill. China's biggest tweak yet—counting absentee residents—should only increase the uncertainty.

—MARA HVISTENDAHL

Mara Hvistendahl is a journalist based in the Netherlands.



Demographer's nightmare? Hordes of migrant workers are hard to enumerate.

tified people using household registration records. Migrants had to have lived in a city for 6 months to be counted, a requirement that made it easy for census takers to miss recent arrivals. The result was an unprecedented rate of undercounting attached to China's official tally. China's last population figure of 1.3 billion includes 22.5 million Chinese thought to have been missed in the 2000 count. Many countries adjust results to account for underreporting and other statistical inconsistencies; on that score, China

From *Science's* Online Daily News Site

Caribbean Coral Die-Off

Scientists studying Caribbean reefs say that 2010 may be the worst year ever for coral death there.

Bleaching occurs when crucial microorganisms leave coral reefs during stress, such as prolonged warming. For example, in 2005, water temperatures off the Virgin Islands rose just 3°C above the average in August but stayed that way until November, bleaching 80% and killing up to 40% of corals on the eastern side of the Caribbean.



Now temperature maps produced by the U.S. National Oceanic and Atmospheric Administration indicate that, since reaching abnormally high temperatures in June, the water has remained hot for longer than in the 2005 episode. And the warming has affected a much broader area of the western and southern Caribbean, devastating reefs in the Netherlands Antilles and off the coast of Panama. Although some wind has mixed and cooled the waters, researchers in the

area say the bleaching is worse than they've seen before, affecting hard corals, sponges, and sea anemones.

<http://scim.ag/bleached-coral>

'Dance Your Ph.D.' Winner Announced

What kind of science makes the best dance? The overall winner of the 2010 "Dance Your Ph.D." contest has proven that it just might be chemistry. On 18 October, at the Imagine Science Film Festival in New York City, a judging panel announced that Ph.D. student Maureen McKeague's dance interpretation of her research on designer molecules had beat out the best Ph.D. dances from physics, biology, and the social sciences. McKeague was also the winner of *Science's* online reader poll.

Just like her research, "this was a group effort that involved our whole lab," says McKeague, who attends Carleton University in Ottawa, Canada. She went home with a \$1000 prize from *Science*, which sponsored the contest. <http://scim.ag/dance-winner>

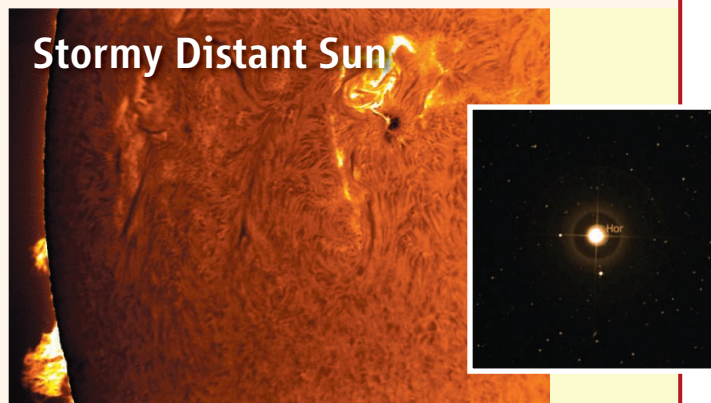


Politicians, Watch Your Grammar

As congressional midterm elections approach in the United States, politicians are touting their credentials, likeability, and policy ideas. But they may be forgetting something crucial: grammar.

Psychologists Teenie Matlock of the University of California, Merced, and Caitlin Fausey of Indiana University, Bloomington, asked 188 university students to read sentences about hypothetical politicians. Half saw statements like, "Last year, Mark was having an affair

Stormy Distant Sun



The planets orbiting Iota Horologii (inset) enjoy only brief respites from their tempestuous sun. The star, located 56 light-years away in the southern constellation Horologium, boasts the shortest starspot cycle ever seen: 1.6 years. The sunspot cycle of our own sun (main image), by contrast, waxes and wanes every 11 years. When sunspot numbers peak, the sun can hurl satellite-frying flares toward Earth. So any residents of the Iota Horologii system—which bears at least one giant planet—presumably experience more frequent outbursts, lead author Travis Metcalfe of the U.S. National Center for Atmospheric Research and colleagues will report in an upcoming issue of *The Astrophysical Journal Letters*. Similar conditions may have confronted Earth as life was struggling to establish itself. At that time, the sun likely resembled Iota Horologii, because the star is young. <http://scim.ag/star-spots>

with his assistant and was taking hush money from a prominent constituent." The other half saw this: "Last year, Mark had an affair with his assistant and took hush money from a prominent constituent."

The difference is one of grammatical aspect: "was having" and "was taking" are known as the imperfect aspect, meaning an event may be continuing. But "had" and "took" are known as the perfect aspect, meaning the event is bounded in time.

The subtle differences had a strong impact: More than three-quarters of students who read the imperfect aspect phrases said they were confident that the politician would not be reelected, whereas only about half who read the perfect aspect phrases felt this way, the researchers report in *Political Psychology*.

In both cases, the perfect aspect—"had an affair"—conveys a sense that the bad deed is in the past, says Matlock. That may make voters more likely to forgive these actions. On the other hand, imperfect phrases such as "was having an affair" suggest that the bad deeds may still be happening and are more relevant to an upcoming election—and hence, that the politician is less electable.

<http://scim.ag/grammar-matters>

Read the full postings, comments, and more at <http://news.sciencemag.org/sciencenow>.

NEWSMAKER INTERVIEW: HAMID AHMED AND SAMIR RAOUF

Iraq Banks on Peer Review to Rebuild Its Research Base

In 2004, a year after the United States and its allies invaded Iraq and overthrew Saddam Hussein, the Coalition Provisional Authority created the Iraqi Nonproliferation Programs Foundation. Although it was supposed to evolve into an agency to fund basic and applied research, its \$47 million allocation never materialized after the new Iraqi government came to power.

Today, only 14 months before the final pullout of all U.S. troops, Iraqi officials are still trying to build a research infrastructure. As part of that effort, a six-member Iraqi delegation visited the United States recently to study the U.S. system. They met with officials at the National Science Foundation, the National Institutes of Health, and the White House Office of Science and Technology Policy and traveled around the country to meet with nongovernmental organizations and universities.

The group's mission is to recommend a science-policy apparatus for the country, once a regional scientific power, that would rest upon a merit-based competitive process of awarding grants. Such a system would replace what Alex Dehgan of the U.S. Agency for International Development in Washington, D.C., calls the primary funding mechanism under the Ba'ath Party dictatorship, "a wave of Saddam's hand."

Last week, *Science* spoke to two leaders of the delegation: **Samir Raouf**, an electrical engineer who is Iraq's senior deputy minister for science and technology, and **Hamid Ahmed**, a physician-scientist who advises the prime minister on education and higher education. Their remarks have been edited for brevity and clarity.

—YUDHIJIT BHATTACHARJEE

Q: What was Iraqi science like under Saddam Hussein?

S.R.: Iraq was once a leader in science and technology among Arab countries. But after Saddam Hussein took power, research and development were almost entirely directed toward military activities; that was to be expected since we went through three wars during the 3 decades of his regime. The wars also had an impact on the economy, reduc-

ing the money allocated to research and to education. Also, the number of Iraqis getting postgraduate degrees from other countries fell to around zero in the second half of the 1980s, and that continued until 2003. That created a barrier between Iraqi science and the rest of the world, creating a kind of isolation that affected the quality and level of science in Iraq.

Q: What is the state of Iraqi science and education today?

H.A.: We're still in a transition period, but a lot of good work has been done. Since 2003, there has been a significant investment in higher education in science. Thousands of Iraqi students have been sent abroad on government scholarships.

S.R.: One of the first decisions was to estab-

and technology can play a role in solving them. Without science and technology, all the solutions to these problems will not be sustainable.

Q: What scientific institutions and agencies does Iraq need?

H.A.: We used to have a national scientific and research council, but the previous regime abolished that. Our mission is to help formulate a body that is responsible for drafting research policy (and funding research), be it within the ministry of science or, because it has wider application, in the ministry of higher education.

Q: Is there anything from the U.S. model that you plan to replicate?

S.R.: The first thing is to finance research through grants that researchers can apply for, irrespective of where they are working. We want to use peer-review and merit based processes; ... this is essential to getting the best people to work on their specialty.

Q: Are there enough Iraqi scientists to have a robust peer-review system?

H.A.: We have enough, ... but if needed, we have thousands of Iraqi expats around the world that we can turn to.

Q: Are there particular areas of science that the government is emphasizing?

S.R.: We want to direct all science and research activities toward development. We have started decommissioning of nuclear facilities, and we are leading an initiative for e-governance in Iraq. Renewable energy is an important area because, although we have petroleum, we also have a sunny environment. Also, we want to focus on environmental research, because the neglect of the environment over the past 3 decades has had a big impact on all aspects of life in Iraq, from agriculture to health.

Q: Five years from now, can we expect to see something like an Iraqi National Science Foundation?

S.R.: *Inshallah* [God willing].

H.A.: We hope so.



New start. Iraqi science officials Hamid Ahmed (left) and Samir Raouf in Washington, D.C.

lish a ministry of science and technology. Most of the organizations that were linked to scientific research and had people working on science and technology were organized under this ministry. But rebuilding science and technology is not an easy task; it will take time.

Q: Are there things the country must attend to before rebuilding science?

H.A.: Yes, security is a priority, and most of the government's efforts and funds are directed toward security. Second priority I would say is services. Because of the wars and sanctions, the infrastructure is devastated; electricity, water, health services are not yet up and running.

S.R.: But all these problems, ... science



Who made them?
Jewelry attributed to
Neandertals might have
been made by moderns.

ARCHAEOLOGY

Reanalysis of French Cave Could Deal Setback to Neandertal Smarts

Neandertals have been on a roll in recent years. Once regarded as brutish and dumb compared to modern humans, our closest cousins are now often credited with “modern behaviors” such as crafting sophisticated tools and symbolic personal ornaments (*Science*, 15 January, p. 255). But new radiocarbon dating at a celebrated site in France could mar this flattering view of Neandertal capabilities. The study, published online this week in the *Proceedings of the National Academy of Sciences*, concludes that jewelry and tools once attributed to Neandertals may be the work of modern humans. “This key site should be disqualified from the debate over [Neandertal] symbolism,” says archaeologist Randall White of New York University, who was not involved in the work. But archaeologist João Zilhão of the University of Bristol in the United Kingdom says that the new study “prove[s] the exact opposite of what [its] authors claim.”

The Grotte du Renne (“reindeer cave”) at Arcy-sur-Cure in central France was excavated between 1949 and 1963 by the late French prehistorian André Leroi-Gourhan, who found 15 archaeological levels. Leroi-Gourhan attributed artifacts in the lowest levels to Neandertals and artifacts from higher levels to modern humans, and later scholars have supported this interpretation. But the middle layers included bone tools, ivory ornaments, and other sophisticated artifacts that Leroi-Gourhan attributed to a culture called the Châtelperronian, which many researchers think was the work of Neandertals. Indeed, Leroi-Gourhan found about 30 Neandertal teeth in the Châtelperronian levels.

Most debates about the Châtelperronian—which began about when modern humans showed up in Europe, 40,000 years ago—have revolved around whether Neandertals developed the culture inde-

pendently or simply copied the behavior of incoming modern humans. But recently, some researchers have questioned whether Neandertals made the Châtelperronian artifacts at all.

In the new study, a team led by dating expert Thomas Higham of the University of Oxford in the United Kingdom reports 31 new radiocarbon dates from the Grotte du Renne using novel methods designed to avoid contamination. The dates, obtained on materials such as bone tools and ornaments made of animal teeth, paint a disturbing picture: While upper layers attributed to modern humans clock in at no older than 35,000 years, artifacts from the Châtelperronian levels range from 21,000 years old, when Neandertals were long extinct, to 49,000 years old, before the Châtelperronian began. About one-third of the dates were outside the expected range.

The team concludes that the archaeological layers must have become mixed over thousands of years, raising the possibility that younger artifacts made by modern humans moved down into lower levels. “The evidence from the Grotte du Renne ought to be viewed with extreme caution,” the authors write. White agrees, pointing out that only one other undisputed Châtelperronian site has produced personal ornaments, and in much smaller numbers, thus leaving the case for Neandertal symbolism on shaky ground.

Zilhão counters that two-thirds of the dates at the Grotte du Renne do fall within the Châtelperronian period and that the outliers could be due to contamination. But Higham, who has been redating nearly 20 other Neandertal and modern human sites in Europe, says the Grotte du Renne is unique in producing such wildly varying dates. “I think this is telling us something,” he says.

—MICHAEL BALTER

ScienceInsider

From the *Science* Policy Blog



The presence of protesters last week at an agribiotech symposium at Huazhong Agricultural University led to an impromptu session between members of the general public and scientists that revealed the public’s deep distrust of—and confusion about—**genetically modified crops**. <http://scim.ag/china-gmcrops>

President Barack Obama has kept a promise by honoring dozens of students for their achievements in **science competitions around the country**. The White House event was part of a national science and engineering festival. <http://scim.ag/sci-fair>

The nations that make up the Intergovernmental Panel on Climate Change have made some changes aimed at improving **how the organization responds to critics**, deals with scientific uncertainty, and handles its day-to-day affairs. <http://scim.ag/busan-ipcc>

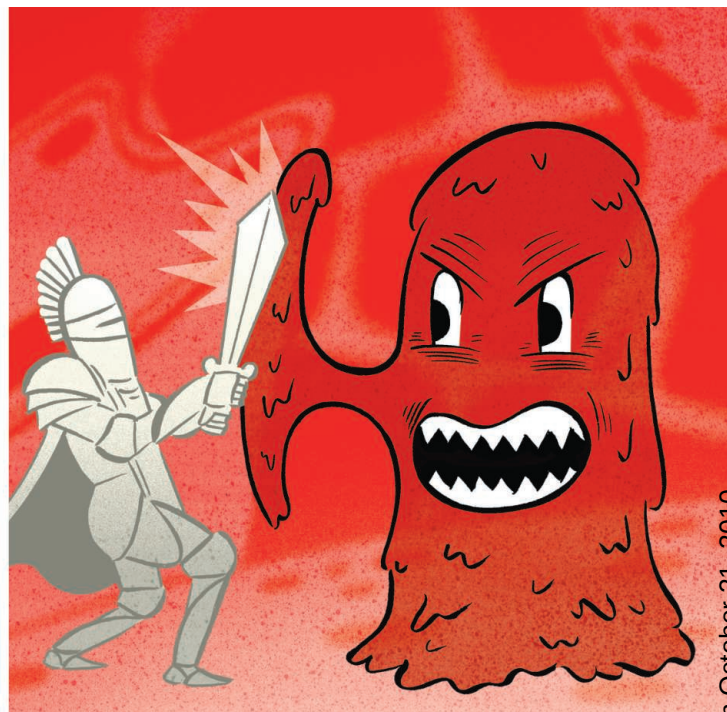
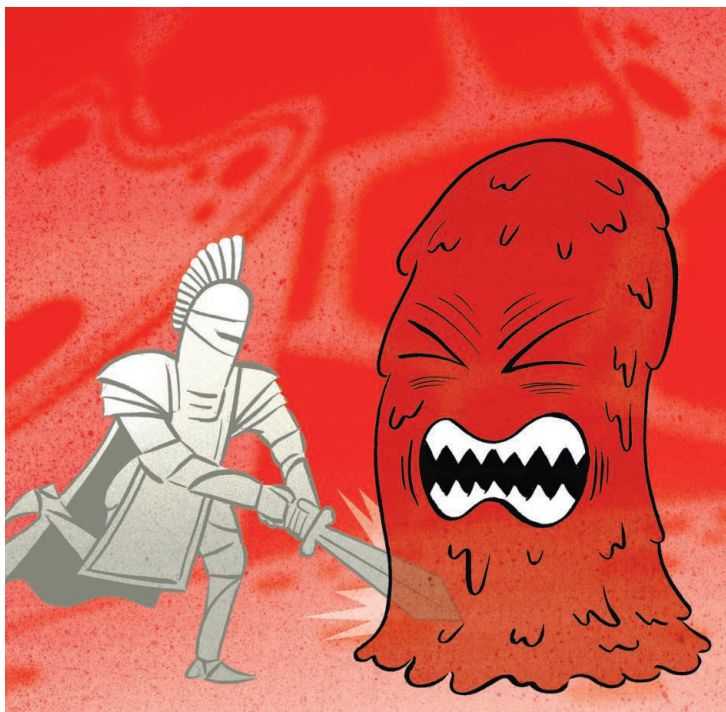
The Nuclear Regulatory Commission is playing politics with the fate of a technical study of the **Yucca Mountain nuclear waste repository**, say congressional Republicans, who add that the Obama Administration’s attempt to end the project preempts federal legislation. <http://scim.ag/report-yucca>

U.S. National Cancer Institute Director Harold Varmus wants cancer researchers to chew on some “provocative questions” as the first step in **tweaking the research agenda** for the \$5 billion NIH agency. <http://scim.ag/cancer-questions>

Food production will need to double worldwide to meet the needs of a growing population. But a new report says productivity will need to increase even faster to avoid causing greater environmental damage to the planet. <http://scim.ag/more-food-report>

The U.S. **national medals** of science and technology, a **research prize** for understanding violence in children, and a new science-writing award were announced last week. <http://scim.ag/STmedals2010>, <http://scim.ag/prize-jacobs>

For more science policy news, visit <http://news.sciencemag.org/scienceinsider>.



Immune Therapy Steps Up the Attack

After years of trying, cancer researchers say they are finally having success enlisting the body's own defenses to destroy tumors

BETHESDA, MARYLAND—On a corner of Steven Rosenberg's desk rests a small, gold figurine representing Sisyphus, its arms straining to push a boulder forward. A gift from his wife when he joined the U.S. National Cancer Institute (NCI) here as chief of surgery back in 1974, it is uncannily apt: Rosenberg has spent 3 decades rolling metaphorical boulders uphill, only to see them tumble back down again.

Rosenberg's specialty is immunotherapy; he tries to harness a patient's own immune system to fight cancer. He and others have seen remnants of immune system attacks on tumors, but the cancer recovers and takes off. Efforts to lend the immune system a hand have raised hopes, leading to large clinical trials of cancer vaccines. But all have flopped. And year after year, nearly all of Rosenberg's patients, recipients of radical experimental treatments, succumbed to their cancer.

Nearly all, that is, until recently.

Slowly, new immune-based therapies are registering successes. In some people riddled

with the aggressive skin cancer melanoma, immunotherapy has not only eliminated disease but also kept it at bay for years. Such outcomes are virtually unheard of in patients with metastatic disease that has spread through the body. Last month, NCI awarded \$14 million to the Fred Hutchinson Cancer Research Center in Seattle, Washington, so it could launch a new national network of immunotherapy clinical trials.

From a patient's perspective, the achievements are still tenuous. Some individuals respond dramatically. But only a fraction are treated successfully—about 15% at most, though some small trials have hints of higher numbers. What excites immunotherapists is that this modest group of responders—the “tail end of the curve”—keeps showing up in recent studies. This year, it appeared in trials of two antibodies used against several different cancers, and in data from Rosenberg's cell therapy recently published or presented at meetings. With more tinkering, cancer specialists hope to shift additional patients

into the responder category and devise more powerful combination treatments. Accustomed to disappointment, they have rarely been so confident.

At the same time, their successes are raising deep questions about where cancer therapy is headed. Integrating immunotherapy into clinical care will pose challenges of its own. Patients may take months to respond, making it difficult to assess whether treatment is helping. Furthermore, some treatments are highly personalized and impossible to administer outside of specialized settings. This makes them extraordinarily expensive. Many specialists wonder whether they can really become part of standard cancer care.

Rosenberg is a believer. “The goal right now is to find things that work,” he says. “When you find things that work, industry finds ways to make it happen.”

Rocky start

One patient has never left Rosenberg's thoughts. As a junior resident in a Boston hospital in 1968, Rosenberg met James DeAngelo, then in his 60s, who had been admitted for gallbladder surgery. Twelve years earlier, DeAngelo had developed a stomach cancer that spread to his liver and was sent home to die. Instead, his cancer spontaneously regressed without treatment—“one of the rarest events in all of medicine,” Rosenberg says now.

One of Rosenberg's first surgery cases became part of an experiment. At the time, Rosenberg recalls, “there was someone else

Reinforcements! After the immune system tries and fails to fight tumors (first two panels), new therapies help these T cell knights get the job done.

cine who focuses on melanoma and kidney cancer. But, he concedes, “for the most part, none of those things really did much.”

Because tumors evolve to prevent the body from recognizing them as foreign, vaccines need to trigger a massive immune response. Most cancer vaccines just haven’t been potent enough, says Jeffrey Weber, a melanoma specialist at the H. Lee Moffitt Cancer Center and Research Institute in Tampa, Florida: “We’ve been giving very wimpy immunizations, in my view.” They may have been ineffective for another reason: Researchers had only a rudimentary understanding of how the immune system and tumors interact. Slowly, that is changing.

Antibody breakthroughs

“The science is now guiding the medicine,” says Jedd Wolchok, a melanoma specialist at Memorial Sloan-Kettering Cancer Center in New York City. “The paths that we are taking are built upon a much more solid understanding of what is going on molecularly.”

Empowering T cells is a key part of the new strategy. One breakthrough came in 1996, when Wolchok’s colleague James Allison reported in *Science* that a protein called CTLA-4 makes T cells less active (22 March 1996, p. 1734). In mice, Allison found that blocking CTLA-4 with an antibody killed tumors. A biotechnology company called Medarex picked up anti-CTLA-4, which now goes by the generic name ipilimumab, in hopes of turning it into a cancer therapy. Wolchok, Rosenberg, and others began testing it in people.

In a small number of patients, the results were dramatic. “I do remember those early days; we were looking at CT scans and saying, ‘Oh my goodness, this thing’s really working,’” says Topalian. In the summer of 2009, the pharmaceutical giant Bristol-Myers Squibb purchased Medarex for \$2.4 billion, gaining rights to both antibodies.

In August 2010, the company and its academic collaborators reported on the phase III results of an ipilimumab trial with 676 melanoma patients. For the first time ever, a randomized trial found that people with stage 4 melanoma benefited from a new treatment. The advantage was modest: Treated patients survived 10 months, on average, compared with 6.4 months for controls. “What is exciting is when you look at the tail of the curve,” says Allison. “Very few patients survive more than 2 years with metastatic mela-

in the hospital who had gastric cancer” and whose blood type matched DeAngelo’s. Following the wild theory that something in DeAngelo’s blood could help this desperately ill man, Rosenberg transfused blood from DeAngelo into the second patient. Nothing happened, and that man later died of his cancer.

But Rosenberg couldn’t shake the memory of that gallbladder surgery, where he ran his hands across DeAngelo’s liver and found no hint of disease. Had DeAngelo’s body fought off cancer on its own?

In some ways, the notion didn’t make sense. Cancer cells are a patient’s own, so why would the body perceive them as invaders? The answers are now clear. Among them: Tumor cells are genetically unstable and pile on new mutations that render them distinct from the host. In 2007, oncologist Bert Vogelstein of Johns Hopkins University in Baltimore, Maryland, and his colleagues reported in *Science* that breast and colon cancers can harbor hundreds of gene mutations—an “unexpectedly high number,” says Suzanne Topalian, a Johns Hopkins melanoma specialist who was not involved in the work. And those mutations “should be recognized by the immune system,” she says. Studies have confirmed it: Tumor cells often display antigens not found elsewhere in the body that prompt immune reactions.

Immunotherapists targeted melanoma because primary melanoma tumors—as

opposed to metastatic ones—are among the few that can spontaneously disappear. Doctors also identified antibodies to melanoma in the blood of patients and a higher incidence of the cancer in those who’d received organ transplants and had suppressed immunity. All of these clues suggested that the immune system engaged in an elaborate dance with the disease.

Given that roughly 8 million people around the world will die of cancer this year, it’s clear that the immune system alone is no match for cancer’s wiles. Robert Schreiber of Washington University School of Medicine

in St. Louis, Missouri, advanced a framework in 2001 that’s often cited to explain why. Schreiber argued that the immune system does go after the tumor initially: He called it the elimination phase.

This may destroy many cancers before they’re detected. But he argued that other tumors develop an immunosuppressive barrier, expressing proteins on their surface that dampen immune attacks. In this equilibrium phase, the tumor and the person with cancer coexist. At some point, the cancer slips into the escape phase, where the balance tips in its favor.

In the past 2 decades, dozens of therapies have tried to stimulate the immune system against cancer, including about 20 vaccines that reached mid- and late-stage clinical trials. “We’ve been excited by every single one of these,” says Mario Sznol, an oncologist at the Yale School of Medi-

Online

sciencemag.org

S Podcast interview with author Jennifer Couzin-Frankel.



noma,” and in this trial, just under a quarter of those treated did. Many still have tumors, however, but their disease is stable and they don’t need treatment, says Allison. Only three out of the 540 who received the therapy were free of cancer altogether.

Just as chemotherapy comes with risks, so does ipilimumab. Side effects included severe diarrhea, colitis, and endocrine disruption; 14 patients died from the treatment. Still, researchers believe that ipilimumab will gain approval from the U.S. Food and Drug Administration (FDA) in the coming months.



shown up in ovarian, prostate, and lung cancer, in addition to melanoma, says Allison. In theory, if a patient’s T cells are reacting to tumor antigens, this approach “can be used for any kind of cancer.”

Narrow success

Antibodies like ipilimumab are valuable, Rosenberg says, but in his mind, they’re not nearly enough. If the cancer doesn’t completely disappear, “everybody dies” eventually, he says. Rosenberg wants a cure, and he is willing to go to great lengths to get it.

He’s testing a different approach at the

goal is to extract these T cells, grow them into the tens of billions outside the body over several weeks, and give them back. To make this work, Rosenberg discovered several years ago that he first needs to destroy a patient’s existing immune cells with high doses of chemotherapy and sometimes total-body irradiation.

Rosenberg’s approach is not an option for many patients. T cells can’t be harvested from those with inaccessible tumors, about one-fifth of melanoma patients. For another fifth, the cells don’t grow well outside the body. Many can’t wait the month it takes to expand cells in the lab. And the pretreatment chemotherapy, as well as a drug given during treatment, is so toxic that most people over 70 can’t tolerate it.

But for those lucky enough to have that precious bag of T cells returned to them, the likelihood of success is impressive. Out of 93 patients with metastatic melanoma who have received the treatment and been followed long-term, 20 saw their cancer disappear completely. Nineteen have remained cancer-free for 3 to 8 years. In another 32, tumors shrank. Nearly all of these patients had failed every available therapy, including, in many cases, ipilimumab.

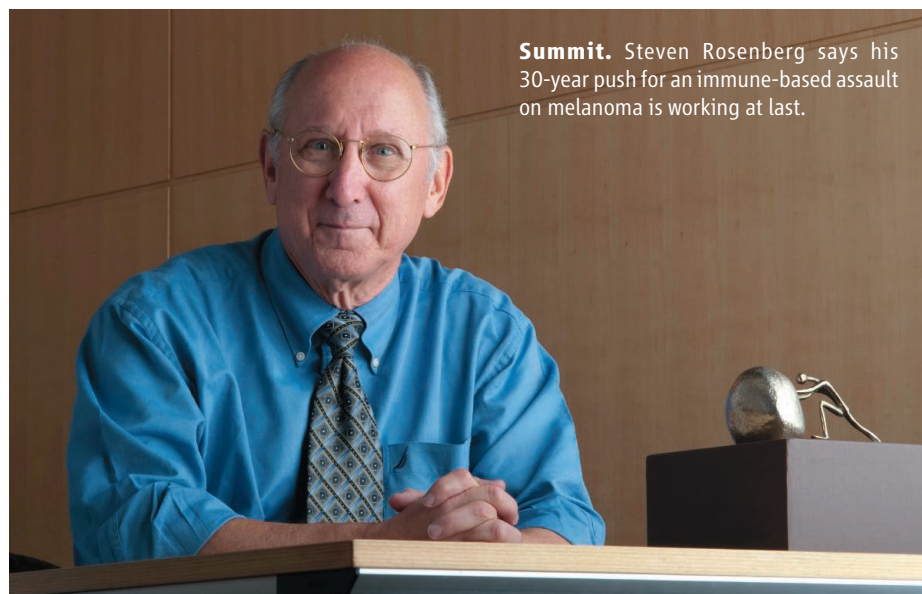
In his office, Rosenberg clicks through a series of CT scans on his computer screen from one of his success stories, a police officer coming in for a follow-up visit. Like most of Rosenberg’s patients, the policeman had metastatic melanoma. Although suffering long-term effects from radiation he received when Rosenberg treated him, he’s been free of cancer since T-cell therapy 4 years ago.

Rosenberg is now trying to get around one big limitation of his strategy: the need for tumor tissue as a source of T cells. He’s experimenting with removing T cells directly from the blood of patients and genetically engineering them to recognize antigens on tumors. This would potentially

open up the therapy to people with all sorts of cancers, especially those with hard-to-reach tumors that can’t be surgically removed for the T-cell hunt. Rosenberg is also beginning to extend his therapy to lymphoma and sarcoma as well as colon cancer.

So far, the few patients treated are doing well.

Several large academic medical centers in Seattle, Houston, and elsewhere have also been working on refining the therapy, called adoptive T cell transfer (ACT). All report roughly comparable success rates, but none is



Summit. Steven Rosenberg says his 30-year push for an immune-based assault on melanoma is working at last.

Medarex had also been working on another naturally occurring protein called PD-1 that dampens immune responses. Whereas mice without CTLA-4 die of immune defects, those without PD-1 are healthier, suggesting that an antibody against PD-1 could have fewer side effects.

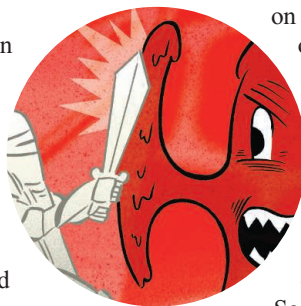
The response rate for anti-PD-1 looks hopeful. Results from a small trial published in July in the *Journal of Clinical Oncology* and additional data presented at a cancer meeting in June suggest that about one-third of melanoma and kidney cancer patients responded—that is, their tumors shrank. “The most amazing thing of all,” says Drew Pardoll of Johns Hopkins, who has been involved in the development of anti-PD-1, is that up to 3 years after treatment, “not a single responder has yet relapsed. ... That’s eye-popping.” Many of them still harbor tumors, but “they’re just sitting there” and not growing, says Pardoll.

Both antibodies are being tested in other cancers. Responses to ipilimumab have

National Institutes of Health (NIH) Clinical Center here, which draws people from all over who are running out of options and treats them free of charge. Rosenberg estimates his budget for immunotherapy at about \$3 million a year, far higher than virtually anywhere else.

On this day he drops in on a cheerful woman in her mid-40s with reddish-brown hair and chunky black glasses. She will be the first person to receive Rosenberg’s immunotherapy for colorectal cancer. If it fails, he guesses she has between 4 and 6 months to live.

Unlike antibody therapy, which is administered intravenously in an outpatient setting, Rosenberg’s method requires hospitalization and a research clinic, at least for now. He focuses on finding T cells activated to attack cancer, usually in tumor tissue. His



A SAMPLING OF ONGOING IMMUNOTHERAPY TRIALS

Therapy	Disease	Trial	Sponsor	Planned Participants
Ipilimumab + radiation	Prostate cancer	Phase III	Bristol-Myers Squibb	800
Ipilimumab	Melanoma	Phase III	Bristol-Myers Squibb	500
Anti-PD-1	Various cancers	Phase I	Bristol-Myers Squibb	124
Ipilimumab and anti-PD-1	Melanoma	Phase I	Bristol-Myers Squibb	50
MAGE-A3 cancer vaccine	Lung cancer	Phase III	GlaxoSmithKline	2270
MAGE-A3 cancer vaccine	Melanoma	Phase III	GlaxoSmithKline	1300
Adoptive T cell transfer	Melanoma	Phase II	National Cancer Institute	135
Adoptive T cell transfer	Blood cancers	Phase I	National Cancer Institute	36

doing as much as Rosenberg at NCI—largely, they say, because of the cost.

Although the results are compelling, ACT “is 100 times as hard to export, to do in a reproducible fashion, and it’s also a lot more expensive than” antibodies, says Pardoll. “Companies essentially have no interest in it. It really right now is a purely academic exercise.”

ACT has taught us a great deal, says Wolchok at Sloan-Kettering—for example, that people with heavy cancer burdens can be helped just by expanding and reintroducing their T cells. But ACT faces daunting hurdles in “being able to produce the cell product for every patient who needs it,” he says. Even in Rosenberg’s lab, the cells don’t always grow, says Wolchok, who has referred patients to the program. Sznol at Yale has looked into setting up an ACT program there, but the logistics have made it too difficult to pull off yet.

Although Rosenberg agrees that companies haven’t invested in ACT, he is frustrated by those who critique its practicality. He has a lab member working full-time with NIH’s blood bank to determine whether it can grow patients’ cells more efficiently. NCI recently began a randomized clinical trial of ACT in melanoma. The hope is that if ACT proves superior, payers might cover its cost, which depends on the protocol but can exceed \$100,000 per patient.

Even doubters recognize that what’s dismissed as impossible in medicine is always changing. “Monoclonal antibodies had the same stones thrown at them 20 years ago,” with everyone questioning their feasibility, says Wolchok. With ACT, “it may just be time for the technology to catch up with the need.”

After much hesitation, pharmaceutical companies have expressed growing enthusiasm for cancer immunotherapy. In April, FDA approved the first cancer vaccine, called Provenge, for metastatic prostate cancer, made by the company Dendreon in

Seattle. Treatment consists of three infusions for a total cost of \$93,000. Many oncologists are underwhelmed by its effectiveness. The vaccine extends survival by 4 months; it does not stop cancer. But just the fact that Dendreon pushed ahead with the vaccine, which is custom-made for every patient, is heartening to some immunotherapists.

Bristol-Myers Squibb has its antibodies—ipilimumab and anti-PD-1—while another pharma giant, GlaxoSmithKline (GSK), is running several large trials of a new cancer vaccine for lung cancer and melanoma. Unlike Provenge, the GSK vaccine is an off-the-shelf mix. It includes an antigen, MAGE-A3, that commonly appears on cancer cells, and an adjuvant to boost immune reactions; introduced together, these aim to stimulate the immune system to go after cells expressing MAGE-A3. GSK is trying to use gene profiling of tumors to carefully pick patients most likely to respond. “We do not want to

worse before they get better,” says Cassian Yee of the Fred Hutchinson Cancer Research Center. “It does take a little bit of insight for the person managing the patient to say, ‘OK, your tumor’s only grown by 10%, 20%—we think that you should continue’ on ipilimumab.

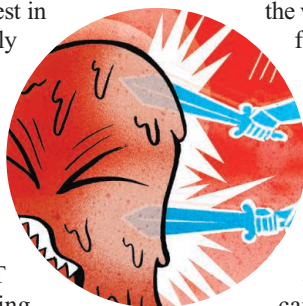
No one yet knows why tumors might grow before they dissipate. Indeed, there’s still much that remains a mystery about how antibodies and ACT behave in the body, how to predict who they’ll help, and how to make them more effective.

A subset of patients might have what Thomas Gajewski of the University of Chicago in Illinois calls the “inflamed phenotype”: They are capable of “making some smoldering immune response against their tumor that you can tip over” in their favor, shrinking the cancer or erasing it altogether. Gajewski estimates that at least 30% of patients fall into this category. He’s looking for ways to coax the other 70% to respond: “How do you make the noninflamed tumors inflamed?” he wonders.

Most cancer specialists believe the solution will come from combination therapies. Like Gajewski, Allison theorizes that immunotherapy is most effective when an immune response is already under way, with T cells activated and tumor cells dying. One way to tip more patients into this category might be by supplementing immunotherapy with direct killing of tumor cells. Bristol-Myers Squibb is running a large prostate cancer trial that combines ipilimumab with a single dose of radiation to do just that. In theory, the antibody might also enhance the potency of T cells given in ACT, says Yee.

Which treatments will go mainstream? “Everybody’s goals are the same: Let’s try to cure cancer patients,” says Rosenberg. But is it reasonable to set up expensive cell growth facilities, as some want NCI to do, especially if they’ll help only a subset of patients? And who will pay? With immunotherapy, no matter which approach you take, says Yee, “you’re going to start off with high expenses.” The hope is that long term, the payoff will be worth it.

—JENNIFER COUZIN-FRANKEL



“The science is now guiding the medicine.”

**—JEDD WOLCHOK,
MEMORIAL SLOAN-KETTERING
CANCER CENTER**

have the clinical efficacy diluted because we don’t select the right patients,” says Vincent Brichard, head of immunotherapeutics at GSK Biologics in Rixensart, Belgium. “This could explain why previous trials have failed.”

Skill and subtlety

As immunotherapy edges into the clinic, it’s likely to challenge oncologists’ expertise. When chemotherapy works, it works quickly; immunotherapy is very different. “You might not see responses right away, and they may get



ASTRONOMY

Radio Astronomers Take Arms Against a Sea of Signals

Beset by interference from telecommunications and radar, researchers are devising workarounds to preserve their view of the long-wavelength universe

The valley around the 100-meter-wide Green Bank Telescope (GBT) in West Virginia is one of the most radio-hostile places on Earth. Transmissions from cell phone towers, broadcast stations, and other devices that could interfere with the telescope's observations of the cosmos are unwelcome here. Two lines of defense shelter GBT (pictured above) and other telescopes that the National Radio Astronomy Observatory (NRAO) operates in Green Bank. First, the Appalachian Mountains block radio transmissions from towers to the southwest. Second, in 1958, the U.S. Congress designated a 34,000-square-kilometer expanse around Green Bank as a National Radio Quiet Zone (NRQZ). Transmitters that could cause radio interference for telescopes at Green Bank are forbidden by law. Cell phones don't work here.

But this haven for radio astronomers is under attack. Over the past decade, Green Bank has faced increasing interference from mobile wireless devices, including Wi-Fi modems and Bluetooth, satellite radio channels like XM and Sirius, global positioning

systems, and other radio sources not covered by the 1958 law. Last year, the switch to all-digital television broadcasting in the United States—a bigger cause of interference than analog signals—added to Green Bank's troubles. Meanwhile, traditional sources of interference such as airplanes and satellites continue their barrage.

Intrusive emissions routinely contaminate data from cosmic radio sources, leaving astronomers to sort through the confusion. In the worst instances, the interfering signals can be strong enough to wipe out several hours of observation in a single stroke, as NRAO astronomer Scott Ransom learned 5 years ago while using GBT to look for pulsars. All of the data from a full night of observing were ruined by what turned out to be a strong Wi-Fi signal from a house nearby. Skiing on Snowshoe Mountain the next day, Ransom found out that the house belonged to the friend he was skiing with: an NRAO engineer who had illicitly installed a wireless modem at home. "He looked very sheepish," Ransom says.

The noise problem at NRQZ is shared by radio telescopes around the world, most of which lack Green Bank's protections. Unfortunately, the more sensitive radio telescopes become to detecting weak signals from space, the more vulnerable they are to humanmade interference. "We're seeing an exponentially growing use of mobile and other wireless devices, even in the relatively desolate areas where most radio telescopes are located," says Andrew Clegg, an official with the U.S. National Science Foundation in Arlington, Virginia, who works to promote the use of the radio spectrum for science. The resulting competition for spectrum, Clegg says, has developed into a significant challenge for radio astronomy.

Astronomers have been developing a slew of solutions to meet that challenge. They include electronics that help radio receivers tolerate data-threatening power surges, filters to avoid frequencies at which interference is likely to occur, and ways of making telescopes and telescope arrays selectively blind to signals coming from certain directions in the sky to cancel out interference from satellites and airplanes.

In addition, astronomers and radio engineers are coming up with new and improved techniques for subtracting interference from data after it has been collected. And observers at Green Bank and elsewhere are also working out human solutions: negotiating with local communities to reduce interference and silence it altogether for short durations to allow key observations.

Lines of defense

The technical fixes start in a telescope's amplifiers. Because radio signals arriving at a telescope's antennae from a cosmic source are usually quite faint, they must be amplified several-fold before they can be processed. For the data to be usable, the amplification needs to be linear—that is, it must multiply the input signal by some constant factor. However, every amplifier has an upper limit beyond which an incoming signal no longer produces linear output. A strong enough interfering signal can ratchet the input past that upper limit. "If the amplification is no longer linear, then all of your data goes bad," explains Bob Hayward, an NRAO engineer.

To guard against such spikes, researchers have been developing amplifiers with higher upper limits, or greater "headroom." New telescopes such as the Low Frequency Array (LOFAR)—a network of 25,000 antennae headquartered in the Netherlands and spread across Europe—have been equipped

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with wide-range digital amplifiers; older telescopes have also been upgrading their electronics against interference.

Once the data have been protected, astronomers must detect and weed out the interfering signal. Traditionally, astronomers had to do much of this manually, painstakingly inspecting the data for anomalous spikes and deleting those portions. Increasingly, that task is being handled automatically and with greater precision.

A lot of interference is transient, such as a burst of emission from an aircraft flying overhead, explains Ger de Bruyn, an astronomer at the National Institute of Radio Astronomy in the Netherlands, which manages LOFAR. The latest digital electronics enable LOFAR's antennae to collect data with very high time resolution—across windows of time that are as short as nanoseconds. This allows LOFAR to remove with great precision those bits of data where interference has occurred, in effect preserving a much bigger percentage of the observed time than would have been possible in the past. “We have the potential to work down to 5 nanoseconds,” de Bruyn says.

There have also been steady improvements in software designed to spot interference, led by groups such as one headed by Dale Gary, an astrophysicist at the New Jersey Institute of Technology in Newark. “The thing we’re taking advantage of is the statistical nature of the varying voltages coming out of the telescope,” Gary says. Voltages from cosmic radio sources, he explains, tend to form Gaussian distributions; those from earthly signals don’t. Gary and his colleagues at Korea Astronomy and Space Science Institute have developed an interference-locating algorithm that quickly determines whether a stream of signals is Gaussian. It’s being used at the Owens Valley Solar Array in California.

Other approaches take weeding out a step further: blocking out whole patches of the sky while continuing to observe the rest. Researchers led by Brian Jeffs, an electrical engineer at Brigham Young University in Provo, Utah, have developed a technique that uses an array of antennae to locate the direction of an interfering signal such as airplane or satellite transmissions. “Before the image is formed, we run the data through a processor that combines the signal from all of the antennas in a way that blocks out the interferer,” Jeffs says. The technique has been tried successfully on a 20-meter telescope at Green Bank; a similar technique is being implemented by LOFAR.

Another simple approach is to filter out interfering frequencies. For his observations of pulsars, NRAO’s Scott Ransom has taken to

using a filter that blocks frequencies between 2 and 2.2 GHz—the range of many wireless devices. “I certainly lose something as a result,” he says. “I could definitely get more sensitivity if I could use the entire band.”

Eternal vigilance

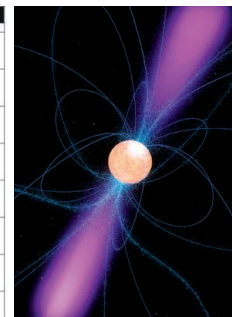
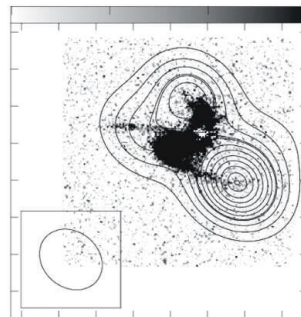
Many radio observatories have spectrum managers whose job includes preempting the destructive effects of interference on their instruments. For example, when a NASA-funded satellite called CloudSat was scheduled for launch in 2005, managers at the Very Long Baseline Array (VLBA)—10 radio dishes laid out across about 8000 kilometers of the United States—flagged it as a threat.

CloudSat was to use a frequency of 94 GHz; VLBA’s receiver covered a range from 80 to 96 GHz. A couple of VLBA antennae pointing straight up at the sky—90 degrees from the ground—could have been fried by CloudSat’s signals. “We changed those antennas to stow at 88 or 87 degrees, like the rest of the array, which precludes the satellite and the VLBA antenna looking straight at each other,” says R. Craig Walker, an astronomer with NRAO in Socorro, New Mexico, where VLBA is headquartered. The potential damage to VLBA from CloudSat had been averted, although astronomers would still have to weed out interference from the satellite’s signals.

Where possible, observatory managers and astronomers have negotiated with nearby communities to reduce or silence interference. At the Giant Metrewave Radio Telescope in Pune, India, for example, administrators were able to get cell phone towers within 20 kilometers of the telescope to shift to a frequency that doesn’t hinder GMRT’s operations. Local officials have also agreed to help keep high-tension power lines free of stray wires, another pesky source of interference. At Green Bank, an Interference Protection Group led by Carla Beaudet has persuaded some local Wi-Fi users to switch to wired Internet connections by offering them free high-quality modems.

“Most people are excited about astronomy and are usually willing to help reduce their transmissions,” says Nissim Kanekar, an astronomer with NRAO in Socorro who has used GBT to study distant galaxies. Two

years ago, Kanekar was scrutinizing a high-redshift galaxy for signs of a certain spectral line that would have helped him probe changes in fundamental constants such as the fine-structure constant over a long period of the universe’s history. The problem was that the line was expected at 855 MHz, within a band of frequencies subject to interference by transmitters of American Electric Power (AEP) located just outside Green Bank’s radio-quiet zone. Kanekar contacted AEP, which agreed to switch off transmitters to allow observations over weekend nights. When that didn’t do the trick, the company obliged with more-extensive shutdowns. “I am still processing the data right now but am



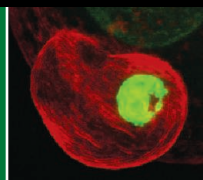
Constrained. NRAO astrophysicists Nissim Kanekar (top) and Scott Ransom have had to deal with interference from satellites and wireless devices in their studies of distant galaxies (above, left) and pulsars (above, right).

quite hopeful that we’ll be able to see the line, which would be fantastic,” Kanekar says.

The wireless and satellite revolutions continue to throw up new challenges. NRAO’s Harvey Liszt notes that automobiles with adaptive cruise control use powerful radar at 76 MHz to maintain a safe distance from other vehicles. “One of these cars could destroy a radio telescope,” says Liszt, who is thankful that the feature has not penetrated the market too far yet. Last year, after Toyota petitioned the Federal Communications Commission to allow higher power radars on cars, Liszt filed a comment alerting authorities to the threat to radio telescopes.

That’s why makers of new radio telescopes like the Square Kilometre Array are drawn to remote, sparsely populated sites: places such as Western Australia and the Northern Cape province of South Africa, whose governments have promised to protect them from interference. Outside such radio-quiet oases, however, astronomers must continue to contend with satellites, cell phones, digital TV, and the rest of the 21st century’s blaring conveniences.

—YUDHIJIT BHATTACHARJEE



LETTERS

edited by Jennifer Sills

Who Pays the Price for Shared Social Responsibility?

IN THEIR REPORT ("SHARED SOCIAL RESPONSIBILITY: A FIELD EXPERIMENT IN PAY-WHAT-YOU-WANT pricing and charitable giving," 16 July, p. 325), A. Gneezy *et al.* conclude that under shared social responsibility (when customers pay what they want instead of a fixed price, and half the proceeds go to charity), "the pursuit of social good does not undermine the pursuit of profit." That is, contrary to the results of Milton Friedman (1), companies' ethics are not competing with company economics. I suggest an alternative interpretation of their results.

In the critical shared social responsibility pay-what-you-want condition, the costs and benefits for the two players (corporations and customers) are very different. As the price paid by a customer increases, the company's direct cost (the purchased photo) doesn't change, whereas the customer's direct cost increases. Meanwhile, the company's direct benefit (profit) increases, whereas the customer's profit (the purchased photo) doesn't change. Furthermore, the company's direct contribution to social welfare (zero) doesn't change, whereas the customer's contribution increases. Therefore, the shared social responsibility pay-what-you-want strategy can be most parsimoniously described as a method for companies to conceal the unequal net benefits from customers while manipulating them to consume more; it creates the illusion that the company is directly contributing to social welfare, so customers are motivated by their own social values to increase company profits through increased consumption. Friedman's definition of corporate responsibility does not conflict with shared social responsibility, and consumers should remain, as Gneezy *et al.* say, "suspicious of the firm's intentions." As Anand and Sen observed, the goal of mainstream economics is "overall wealth maximization" that ignores "social justice and human development" (2); this goal does not optimize social welfare (3).

More important, assumptions about welfare, consumption, and altruism underlying the authors' conclusion contradict two widely accepted observations: (i) Biophysical limits mean that increasing current global consumption levels often reduces general social welfare (4, 5), and (ii) the capacity for moral behavior is evolutionarily adaptive (6), so humans will contribute to social good at net direct cost (7). Therefore, shared social responsibility in this time of global economic, environmental, and social crises may require social (and business) structures that encourage widespread altruistic behavior that is not dependent on increasing physical consumption (8, 9).

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Response

IN OUR INTRODUCTION OF THE CONCEPT OF shared social responsibility, we contend that it can help create value. Cleveland argues that shared social responsibility merely allows companies to conceal the fact that they are profiting from the customers' social responsibility while manipulating them to consume more (thus resulting in unequal net benefits). We agree that shared social responsibility increases consumption, and consider it a crucial observation. Shared social responsibility can increase customer spending, and it is essential to assure that revenue would benefit all parties—charity and customers certainly, but also the company itself. The last element is essential: If companies lose money by using shared social responsibility, they would simply not use it.

With that idea in mind, it is important to consider the issue of concealment. Cleveland gives a plausible account of the differential costs and benefits afforded the company and customer, but he does not consider that the company entirely and asymmetrically assumes the risk. Under shared social responsibility, firms voluntarily expose themselves to the possibility that consumers might pay little, or nothing, in exchange for the good. For example, in the field experiment we reported, marginal costs were close to \$1 per photo. Suspicious customers could have paid \$0 and inflicted a large loss on the company. It is precisely this vulnerability that signals the integrity of the company to its customers, who in turn might increase



Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.



Surviving rejection

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The exposome

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purchase rates or purchase prices. A firm that tries to take advantage of its customers would experience an immediate and substantial loss.

We also agree that “overall wealth maximization” is a goal, and that “optimiz[ing] social welfare” is a more problematic objective. However, Cleveland’s argument about biophysical limits neglects an important aspect of shared social responsibility. He is right to point out that shared social responsibility does not increase the sum of money for the players, but that does not mean that it fails to increase their utility in general. By allowing customers to buy desirable products and simultaneously donate to charity, their utility increases relative to simply buying the product for the same price (*1*). Giving to charity is not a mistake, and it could

benefit people. To the best of our knowledge, there is no biophysical limit to increasing the utility of the players, and shared social responsibility seemingly can increase it for all players: the customers, the charity, and even the company itself.

Shared social responsibility is a tool that helps make corporate social responsibility efforts (donating a percentage of a fixed price to charity) more effective. This also means that shared social responsibility may benefit the company’s bottom line. In our view, it is acceptable, and even desirable, for a company to be profitable. Shared social responsibility provides a tool to increase the company’s well-being in addition to the well-being of its customers and society in general.

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Nuclear Waste: Thorium’s Potential

IN THEIR POLICY FORUM “NUCLEAR WASTE: Knowledge waste?” (13 August, p. 762), E. A. Rosa *et al.* overlook a possible solution to nuclear waste: alternative fuel cycles, particularly the Thorium Fluoride, Molten-Salt Reactor (Thorium MSR).

The use of Thorium as a fertile reactor input has the potential to greatly reduce high-level reactor wastes (*1*). (Thorium-232 is bred by the reactor’s internal neutron flux to Uranium-233, which is then efficiently fissioned by another neutron. A small proton accelerator can also do the breeding. U233 is unnatural, because of a short half life, but fissions more easily than the U235 used in typical reactors.) Adopting the MSR

CORRECTIONS AND CLARIFICATIONS

Reports: "Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes" by J. Rodrigues *et al.* (10 September, p. 1353). The first sentence of Fig. 2 should read as follows: "Effect of immune priming with *Plasmodium* on commensal bacteria, hemocyte populations, and gut-associated hemocyte-specific antiplasmodial mRNAs."

Random Samples: "Dolphin spray yields DNA" (3 September, p. 1131). Researchers compared DNA from dolphin spray to DNA from dolphin blood, which was collected during routine medical procedures, not from biopsy darts.

Reports: "Loss of DNA replication control is a potent inducer of gene amplification" by B. M. Green *et al.* (20 August, p. 943). Because of an error in reference processing, reference 15 (p. 946) incorrectly included an additional author. The correct reference is "15. C. Payen, R. Koszul, B. Dujon, G. Fischer, *PLoS Genet.* 4, e1000175 (2008)." The reference has been corrected in the HTML version online.

Reports: "Increased mutagenesis and unique mutation signature associated with mitotic gene conversion" by W. M. Hicks *et al.* (2 July, p. 82). Because of an error in reference processing, reference 14 (p. 84) incorrectly included an additional author. The correct reference is "14. C. Payen, R. Koszul, B. Dujon, G. Fischer, *PLoS Genet.* 4, e1000175 (2008)." The reference has been corrected in the HTML version online.

would further reduce waste by orders of magnitude (1, 2)—there is no solid fuel or refueling waste, and all fissiles entering the salts are consumed.

This is not a new idea. Edward Teller, long a proponent of nuclear power, suggested we convert to the MSR for civilian power starting in 2002 (3). Earlier, Alvin Weinberg's team at Oak Ridge National Laboratory developed and ran a 7-MW demonstration reactor safely for more than 4 years (4); it was defunded because it didn't fit narrow needs of the Cold War (5). Anthropologists refer to such institutionalized forgetting as "cultural attrition." We can't afford that foolishness, given the problems looming over us today [e.g., (6–9)]. The scalability and safety of the Thorium MSR meets not only inexpensive, emissions-free power needs, but freshwater needs and nonproliferation goals worldwide. And, it can sustain U.S. leadership in world trade and technology.

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Nuclear Waste: Progress with Public Engagement

AS CO-CHAIRMEN OF THE BLUE RIBBON Commission on America's Nuclear Future, we appreciate the comments offered by E. A. Rosa *et al.* in their Policy Forum "Nuclear waste: Knowledge waste?" (13 August, p. 762) regarding the need to consult extensively with social scientists in the conduct of the Commission's deliberations.

E. A. Rosa *et al.* stress "the importance of engaging impacted publics at the beginning of policy planning and projects." We agree. Thus far, the Commission and its subcommittees have visited and heard from the communities surrounding the Hanford nuclear reservation in Washington State, the Idaho National Laboratory, and the site of the shut-down Maine Yankee nuclear power plant. We have also heard invited testimony from state and local government officials and community groups from New Mexico and Nevada. We have invited federal, tribal, and industry officials and a variety of national-level



nongovernmental organizations to appear before the Commission or its subcommittees.

This engagement with impacted publics has benefited us greatly and has highlighted both the diversity and depth of public concern about nuclear waste management. It has also served to broaden the scope of the important questions the Commission seeks to answer as we conduct a comprehensive review of policies for managing the back end of the nuclear cycle. To build upon the information received thus far from hearing public concerns, the Commission has invited leading social scientists to participate in recent meetings of the full Commission and of Commission subcommittees.

Although we agree with many of the comments Rosa and his colleagues offered the Commission, we take issue with their characterization of the Commission's efforts at transparency and inclusiveness. The steps we have taken to ensure transparency far exceed the requirements of the Federal Advisory Committee Act (FACA). For example, we have opened subcommittee presentation sessions to the public, provided live webcasting of full Commission and subcommittee open meetings, extended public comment sessions far beyond what FACA requires, and funded the participation of community representatives in the Commission's proceedings. Rosa *et al.*'s description of this process as a "pro forma approach—where the emphasis is on meeting formal requirements, not the needs of the public" strikes us as based on incomplete information. In fact, we have implemented most of the recommendations on transparency and inclusiveness provided by Rosa and his colleagues in a 25 March 2010 letter to the Commission (1).

The Commission will continue to hear from leading social scientists in the coming months and we are confident their input will play an important role in informing and refining the Commission's recommendations.

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Response

CANNARA RECOMMENDS THE ADOPTION OF the Thorium Molten-Salt Reactor. We urge caution. The nuclear industry has never fully realized safety and other promises. Grimes and Nuttall (1) explain that the fissile Uranium-233 produced by the slow

neutron capture of Thorium-232 “is difficult to extract and handle, because it is produced together with other highly radioactive isotopes, and the performance of thorium fuels is not well understood. The proliferation resistance credentials of the thorium fuel cycle deserve greater scrutiny but appear promising.” These are precisely the kinds of uncertainties and risks that should be part of a wider public discourse about energy choices.

We applaud the commitment of the Blue Ribbon Commission on America’s Nuclear Future (BRCANF) to exceeding the Federal Advisory Committee Act’s (FACA’s) legal requirements, as described in the letter from Hamilton and Scowcroft. However, past research and our own experience in conducting FACA processes show the law to be a minimal standard, suited to hearing prepared (and sometimes strategic) comments from professionals. Such testimonies cannot reveal the depth of distrust among culturally and historically disenfranchised individuals. Hearing public concerns is not the same as engaging with the public and making them part of deliberations. The response to the tritium leak at Brookhaven National Laboratory in 2002 provides a good example of using effective engagement, supported by

social science knowledge, to resolve conflict with the public (2). By focusing on procedural mechanics (e.g., going beyond FACA), the BRCANF does not address the deeper issues of how problems are framed and how options are deliberated. Listening to social scientist explanations is no substitute for carefully conducted research and active outreach to citizens for whom the act of communication may be as important as the communication content.

We argued for social science membership on the BRCANF or its subcommittees because we believe it is important to provide social science expertise on a continuing basis. This structure will ensure the implementation of effective deliberation techniques that engage the public early and continuously—not just to communicate their views but to give them a meaningful role in the decision-making process. As the BRCANF learned at its meetings, social science input has played a constructive role in the design of nuclear waste management planning in Europe (3) and Canada (4). To achieve similar success, the Commission must meaningfully involve the public and the social sciences. The efforts cited in the BRCANF letter will create neither the social trust nor the trustworthiness needed to solve the waste problem.

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HISTORY OF SCIENCE

A Naturalizer's Vision

Robert E. Kohler

Steven Shapin has for some 30-odd years been a leading contributor to what he calls the “naturalizing” of science: the movement among historians, sociologists, and philosophers—and some scientists (think of “Lucky Jim” Watson)—to see science as it is actually carried on and lived rather than as it is ideally portrayed by its official watchdogs and promoters. Naturalizers have worked to deconstruct the notions that science is a uniquely true way of knowing the natural world, that scientists constitute a special kind of knowers set apart by their possession of personal virtues or a universal “scientific method,” that science is best when it is “pure” (i.e., independent of economic and cultural milieus), and that we trust science because we know it to be a true mirror to nature. Naturalizers offer us instead a vision of science as a cultural activity that is an integral element of the societies in which it is practiced, and whose basic mores and conventions it shares. To understand the science of a time and place, one must understand the society whose way of knowing it is.

These efforts, largely successful, have been seen by some as hostile to science—Shapin jokingly refers to naturalizing as “lowering the tone” of history of science—but they are quite the reverse. That is clear from the opening chapters in *Never Pure*, a wide-ranging sample of Shapin's essays. Knowledge of any subject becomes more robust and secure when it is naturalized. Evolutionary biology, physiology, and historical geology became more secure when (in the early 19th century) they were detached from outmoded religious and literary foundations and rebuilt on the basis of empirical facts and theories derived from facts. The same holds for accounts of science itself when they are anchored not in official ideals but in observed behavior. We could say (though Shapin might not) that the naturalizers have made the study of science scientific.

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Shapin as naturalizer stands out in several ways. One is his exceptional range of subject matter: from the new institutions and cultures of modernizing science in early-19th-century Edinburgh and industrializing England, to the experimental virtuosi of 17th-century London, to industrial scientists and biotech-entrepreneurs in 20th-century America, to, most recently, the cultural history of dietetics from Aristotle to Robert Atkins. Another is Shapin's balance of history and social science. He never bogs down in the historical minutia of events and discoveries, nor does he indulge in solipsistic abstractions of pure theory. His sociology is densely empirical, and his history has analytic leaven. This rare ability may reflect his

diverse professional experience in genetics and plant biology, history and sociology of science, and science studies (a field that yokes together history, philosophy, and sociology).

Shapin has contributed an exceptional number of influential and robust concepts to the naturalizing project, especially in his studies of the early modern period. He is a graceful and engaging essayist, and the ample selection of essays

in *Never Pure* (which omits, alas, the crucial work on Victorian science) affords an excellent basis for reflecting on what he has had to say about the life of science.

The two essays in the section “Places and Practices” reflect one of his best-known themes, the constitutive role of physical and social place in making knowledge. Though we tend to think of science as placeless (nature speaking, not us), its authority always derives from place. To make this case, Shapin ferrets out the actual sites where Robert Boyle carried out his pneumatic experiments (mostly

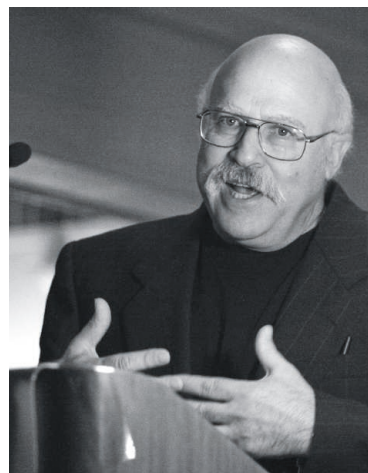
in his several domiciles) and shows how the conventions of experimental philosophy (its rules of access and participation, authenticating, and credit) were those of a gentleman's

residence. He likewise reconstructs the physical trajectory of experiments (and of actual air pumps) from the private spaces of Boyle's and Robert Hooke's dwellings into the public space of the Royal Society's meeting room—where experiments perfected in camera were demonstrated and warranted before witnesses with parliamentary rules of access, discourse, and decorum. Tracing the actual spatial history of scientific objects and knowledge claims thus reveals the basic epistemic rules

of making knowledge. And these rules turn out to be adapted from those of the gentlemanly civil society in which Boyle and his fellow virtuosi lived. The social logic is the same, though not the cultural particulars, in the science of our own middle-class society of technical occupations and careers. [Note the spatial logic lurking in the meritocratic imperative to go public (or perish) and in our modern devotion to placeless places like labs, airports, and conference centers.]

The four chapters in “The Scientific Person” fruitfully develop the linked concepts of identity and trust. Science works on trust and must—because only a tiny fraction of a society knows anything at all of science's substance and because any one scientist knows but a tiny corner of a vast world. So trust cannot derive from firsthand knowledge and must be constituted indirectly, by creating social categories or identities that are accessible to all and that designate certain types of knowledge makers as trustworthy witnesses of nature. As Shapin puts the point later in the book, people trust science not because they know it but because they know where to look for it: they know and trust the type, not the man or woman. How scientific identities are formed and function thus becomes a central concern in science studies.

As Shapin demonstrates, identities and trust are created out of the varied materials of existing social roles. In the early modern period, for example, knowledge makers included pharmacists, alchemists, schoolmen or professors, engineers and mechan-



“Lowering the tone.” Shapin delivering his History of Science Society Distinguished Lecture (Pittsburgh, 2008).

Never Pure

Historical Studies of Science as if It Was Produced by People with Bodies, Situated in Time, Space, Culture, and Society, and Struggling for Credibility and Authority

by Steven Shapin

Johns Hopkins University Press, Baltimore, MD, 2010. 564 pp. \$70. ISBN 9780801894206. Paper, \$30. ISBN 9780801894213.

ics, and gentlemanly virtuosi. Trust was distributed unevenly among these established types: some were suspected of being slaves to economic need or sectarian philosophical systems and were thus not to be trusted; while gents, because they were independent, could be assumed trustworthy—that was the social logic, if not the literal truth. So even though a vanishingly small number of gentlemen ever did natural philosophy, the identity embodied by Boyle gave dignity and authority to the natural philosophy of that time and place. Other times and places have favored other high-status identities: court philosophers in early-modern Italy (Galileo), public men of science in Victorian Britain (Darwin, Huxley), and academic discipline builders in Germany (Helmholtz, Liebig). In our own world of industrial capitalism, social-engineering states, and knowledge-based occupations, we trust “the expert.” The particulars vary, but the social logic of Shapin’s analysis works for all.

These are but three of the many interesting themes that Shapin develops in the volume’s essays. I haven’t the space to discuss other insightful pieces, such as the chapter on the epistemics of proverbs and a trio on the relation between expert and lay or vernacular knowledge in dietetics—a subject in which everyone, and no one, is an expert. But enough has been said, I hope, to give readers unfamiliar with Shapin’s work some sense of his distinctive ideas and why his essays might be worth reading. Think of the author as a social anthropologist of our Western way of knowing, ransacking sources for the facts of real behaviors and beliefs and from these data inferring the operating principles that have made science in its varied embodiments so large and successful.

Yet these are unlikely to be the final words on the naturalizing of science. One becomes aware, reading these essays, of a certain tendency to throw out babies with bath water: for example, the ideas of “scientific method” and “pure science.” In deconstructing these notions, Shapin takes as his targets their most extreme and unrealistic versions, creating straw men that are easy to discredit. He does not enquire, as a social anthropologist should, whether these apparently irrational concepts were in fact quite reasonable and useful at the time and in the place where modern career science was taking shape, in the systems of state education and the political economies of industrializing Europe. I think there was in fact a scientific method—just not what its later advocates and deconstructors said it was. I also believe that “pure science” realistically describes the science

most valued in the first, academic, market for career scientists.

Likewise with the idea that science, as the social philosopher Ernest Gellner provocatively and somewhat mysteriously put it, is “roughly ... the mode of cognition of industrial society” (1). In the volume’s last essay, “Science and the Modern World,” Shapin empties this idea of any real meaning by setting up straw-man versions (e.g., that scientists have both knowledge and the power to use it) that are easily dismissed. Yet Gellner was almost certainly on to a deep truth that calls for empirical historical enquiry, not dismissal. The historian Richard Westfall, whom Shapin quotes disapprovingly, came close to the truth when he observed that science “has shaped most of the categories in terms of which we think” (2). It thus begins to appear

that the revisions of the naturalizers were not as naturalistic as they once seemed; and if this is so then it may be time to revisit their work and retrieve some of what has been cast aside in our fitful progress toward a fully realistic view of what science has been and is.

Reading the essays collected in *Never Pure* made me want to get on the phone and converse and argue with their author—testimony to their lasting power to inform and provoke. Readers unfamiliar with Shapin’s ideas may be similarly inspired.

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10.1126/science.1196506

EXHIBITION

The Agora of Knowledge

Manfred D. Laubichler

An anonymous woodcut reproduced in Camille Flammarion’s 1888 book *L’atmosphère: météorologie populaire* (1) depicts a medieval pilgrim breaking through the sky and glancing at the heavenly spheres beyond. This image, a 19th-century composite of several older motifs, has become an icon for modern science’s quest to expand our understanding of the world. It is also reflected in

Mark Dion’s installation at the center of the exhibition *WeltWissen* (World Knowledge) at the Martin-Gropius-Bau in Berlin.

Dion’s installation consists of a gigantic curved screen of shelves cutting through the museum’s central courtyard. Its curvature suggests the surface of a sphere or, rather, a small section of one. Visitors can approach this boundary of knowledge from either direction, the inside or the (opaque) outside. On the shelves are some 400 objects representing 350 years of scientific research and collecting in Berlin—from the skeleton of Frederick the Great’s favorite horse to museum cabinets, fossil specimens, busts, and archaeological and technological artifacts.

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Dion’s installation also takes a cue from Gottfried Wilhelm Leibniz’s concept of a *Theatrum Naturae et Artis* (Theater of Nature and Art) intended to transform the Wunder- and

Kunstkammer of earlier periods into the foundation for organized scientific and scholarly pursuits. Not surprisingly, Leibniz and his vision also appear at the beginning of the exhibition’s historical part, which

documents the development of more than 300 years of scientific and scholarly activity in Berlin. In Berlin, 2010 has been celebrated as the “year of science” because it marks several important anniversaries: the Charité, Berlin’s famed hospital and medical school, was founded 300 years ago; the University of Berlin–Humboldt University opened its doors in 1810; the Kaiser Wilhelm–Max Planck Society was established in 1911; and, last but not least, on 3 October 1990 Germany reunified, thus beginning a complex process of merging two separate scientific traditions. As this part of the exhibition shows, all these events have transformed the pursuit of knowledge in multiple ways.

As a city, Berlin offers a unique opportunity for a case study of how the development of science is intertwined with varied historical, political, and social contexts. Science

WeltWissen [World Knowledge]
300 Jahre Wissenschaften in Berlin
Jochen Hennig, Curator
Martin-Gropius-Bau, Berlin.
Through 9 January 2011.
www.weltwissen-berlin.de

in Berlin began in 18th-century Enlightenment ideas of reform. The city subsequently became the incubator for the Humboldtian worldview, the idea of the modern university, and the integrated research and technology enterprises that enabled the scientific prominence of Wilhelminian Germany in the late 19th and early 20th centuries. The latter have become the model for many similar efforts around the world. And the 20th century brought rapid successions of glory and fall,

Leibniz sphere of knowledge, while the exhibition's second part, a set of 11 Wissenswege (pathways to knowledge), breaks through its boundary and shows how science and scholarly pursuits lead us into new and uncharted terrain. The design of WeltWissen thus illustrates its central concept; scientific and scholarly pursuits are rooted in specific historical and local circumstances, but they continuously expand the horizon of knowledge outward in two ways: through the systematic and orderly collection of facts in museums, scholarly editions, and synthetic accounts; and through the unpredictable and anarchic practices of experimental and theoretical research that transcend well-established paths.

Each of the 11 pathways to knowledge represents a scientific practice. Using a treasure trove of artifacts, Jochen Hennig and his fellow curators illustrate how new knowledge is created and transmitted. Their task was made all the more complicated because they understand knowledge to

be the product of Wissenschaft, that uniquely German concept that unites the humanities, social sciences, and natural sciences. Given the diversity and complexity of these pursuits, the curators cannot be congratulated enough for finding a structure that lets visitors participate in the fascination of the creative processes of science and scholarship.

The objects on display are informative. For instance, those in the section on Entwerfen und Verwerfen (sketching and tossing aside) allow us to follow the thought processes of Einstein, Leibniz, Walter Benjamin, Robert Remak, and others as they struggled with ideas in their notebooks. We see firsthand how thoughts are illustrated with sketches, crossed out, tossed aside, and revised and how conclusions are finally reached. This truly is science in action.

The next room, devoted to experiments, lets visitors follow some Berlin discoveries. They can study the experimental systems of Emil du Bois-Reymond and how these instruments advanced our knowledge in neurophysiology or how Robert Koch used the whole city as a laboratory for testing his treatment against tuberculosis. But arguably the most eye-catching object in this room is a simple wooden table with some inconspicuous instruments and electrical wires.

One can't help but wonder how it was possible that Otto Hahn and Lise Meitner discovered nuclear fission with such an unassuming experimental design.

Other pathways to knowledge that are presented include travel (many scientific expeditions originated in Berlin), collecting (the full scope of the reunified scientific collections in Berlin is only now gradually emerging), calculating (documenting the route from Leibniz's calculus to supercomputing), and visualization in its many forms. The exhibit also explores the importance of the social practices of science, such as interpretation and reinterpretation, arguing, corresponding, and cooperating.

Cooperation is one link that connects this Berlin-centered exhibit to the scientific world at large. One sees that cooperation—frequently mixed with competition—has been essential to almost all of the events and discoveries presented in WeltWissen. This inevitably raises the question about the future of science, both in Berlin and beyond. One important answer is found in a corner of the room devoted to “Cooperating and Corresponding.” The Berlin Declaration on Open Access to Knowledge in the Sciences and Humanities (3), sponsored among others by the Max Planck Society, is a reaffirmation of an old value, that knowledge is freedom. Therefore, it should be open and freely accessible to all. The Berlin Declaration represents a vision that stands at the beginning of a new global and digital area of science in the public. It is thus a direct descendant of the 17th-century idea that Berlin should become a Sophopolis, a city of wisdom and knowledge.

Broad and inclusive as it is, the WeltWissen exhibit, however, is only the beginning of engaging the public with science and scholarship. The plans for the Humboldt Forum are now well developed. It will be housed in the Stadtschloss (the reconstruction of which is scheduled to begin in 2014) at the center of Berlin and bring together the collections and libraries of various institutions, as well as provide space for special exhibits and a public forum. Its central mission is to make scientific research transparent and to foster a much-needed public dialogue. After centuries of struggle, with many highs and lows, Berlin seems finally ready to live up to its 17th-century calling.

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10.1126/science.1198260



Tabletop fission. The apparatus used by Otto Hahn and Fritz Strassmann in their 1938 experiment.

catastrophe and rebuilding, noble pursuits and crimes against humanity, and separation and reunification.

The exhibition's historical section illustrates these episodes in a richly contextualized way by presenting well-chosen artifacts and documents. Individual events, some better known than others, gain new meaning by virtue of being part of this *longue durée* vision. Of special interest here are the past 20 years of Berlin and German science. After reunification, two different systems were merged at rapid speed. Although on the one hand, this process brought together Berlin's invaluable collections—symbolized in the exhibit by the original score of Beethoven's Ninth Symphony, parts of which were in the East (after a post–World War II odyssey through Poland) while others reached West Berlin from Tübingen (where they had been stored to escape the bombings)—for many other aspects of science, reunification was a missed opportunity. As the historian Götz Aly noted at the time: “Germany must be a very rich land, if it can afford to disregard so many scientists” (2). It is now clear that the dismissal (*Abwicklung*) of many East German scientists and scientific institutions was a case of Prussian efficiency gone awry.

History takes up the inside of the Dion-

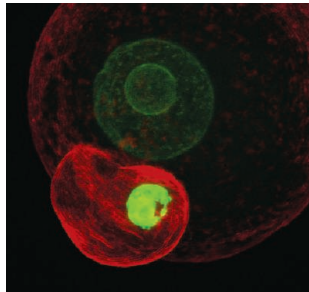
RESEARCH FUNDING

Politics and Parthenotes

C. Tingen,^{1*} S. Rodriguez,^{1,2*} L. Campo-Engelstein,^{1,2*} T. K. Woodruff[†]

On 23 August 2010, federal judge Royce Lamberth ruled that President Obama's 2009 executive order expanding federal funding of human embryonic stem cell (hESC) research violated a federal law, the Dickey-Wicker Amendment (DWA), which prevents the funding of research that destroys embryos (1). This congressional prohibition defines a human embryo as "any organism not protected as a human subject" that was "derived by fertilization, parthenogenesis, or any other means from one or more human gametes" (2). The situation is changing rapidly, with court decisions pending and the possibility of legislation that may, for example, exempt hESCs from DWA provisions (3). In danger of being overlooked is the wording about parthenogenesis, which threatens a separate, but potentially fruitful, area of research.

Parthenotes are cells derived by parthenogenesis, the process in which eggs become activated to begin dividing without fertilization. They appear to have been added to the DWA in response to recommendations issued in 1994 by the National Institutes of Health (NIH) "Human Embryo Research Panel," which supported federal funding for research using parthenotes (4). Although the panel treated parthenotes as distinct from embryos in its recommendations, the DWA, annually passed since 1996, conflated the two. They are not the same. Parthenotes contain genetic material from only one source. In contrast, embryos are created through fertilization and contain genetic material from two genetically dissimilar cells. No viable human parthenote has been reported. Scientists can create human parthenotes in the lab by activating eggs through chemical stimuli that mimic fertilization, but studies in other mammals indicate that, without the required genetic imprinting, further development is ruled out (5–7).



A mouse parthenote. Although its early development mirrors that of a normal embryo, a parthenote contains only egg-derived DNA (green) and cannot become viable.

The potential benefits of research using parthenogenesis are broad. For example, parthenotes could be used to study fertility preservation options for girls and young women whose cancer treatment may render them infertile. A means to store ovarian follicles was developed, with the hope that if these women decide to have biological children, the follicles might be matured to produce eggs that could then be fertilized in vitro (8, 9). But the DWA would prevent NIH-funded researchers from determining whether the eggs so formed could be activated to become a parthenote, thus blocking the testing of whether eggs developed from frozen follicles are truly mature and viable. A distinct advantage of using parthenotes is that they can be activated synchronously, making it much easier to study the timing of activation (10).

Parthenotes are also useful to study cancer and benign gonadal tumors, which are sometimes caused by spontaneous egg activation. For example, ovarian teratomas are believed to occur from spontaneous parthenogenesis of eggs and represent the most common pediatric ovarian tumor (11). Even benign teratomas can cause severe abdominal pain and ovarian torsion, requiring corrective surgery (12). Rarely, these benign teratomas undergo malignant transformation, leading to several cancers, including squamous cell carcinomas and melanoma (13). Studying the process of early egg activation could disclose what goes awry in these tumors.

Scientists have now created ESCs from mouse, monkey, and human eggs (14–16). However, whether hESCs derived from embryos, induced pluripotent stem cells, adult stem cells, or parthenotes will be more or less useful in any particular therapeutic application is not yet known (17). Research using parthenotes avoids many ethical concerns raised by embryo research because, according to a variety of criteria, parthenotes are less likely than embryos to be classified as persons or as deserving the same level of ethical status. For example, one of the most

Broad restrictions on funding for human embryo research can stymie work that was unnecessarily precluded by a flawed U.S. law.

conservative definitions for the beginning of human life is the moment of fertilization (18). Because parthenotes have not been fertilized, this definition precludes their personhood. Others have argued that it is not fertilization that is the key to personhood but rather what occurs at fertilization: the emergence of a unique genetic constitution (18). However, parthenotes are genetically identical to the egg from which they formed and lack normal imprinting patterns. Some who do not believe embryos constitute persons may still object to using them for research because of their potential to become persons. However, all evidence indicates that parthenotes have limited potential and do not survive past an early phase of development. Use of parthenotes does not, however, alleviate the moral dilemmas of egg donation for other than reproductive purposes.

Our society is missing the opportunity to benefit from research that involves parthenogenesis. We support federal funding for parthenote research according to the guidelines recommended by the 1994 NIH panel (4) and call for removal of parthenotes from the DWA, if not repeal of the amendment in its entirety.

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Flipping the Light Switch

Nina Vogt and Claude Desplan

The behavior of animals is flexible and can change dramatically in response to the environment, nutritional state, or even age, among other factors. However, the neural basis of how external and internal cues modify innate behavior is not clearly understood. For example, the fruit fly *Drosophila melanogaster* is inherently either attracted or repelled by vinegar depending on its concentration (1). And age changes the innate light avoidance behavior of young *Drosophila* larvae into light preference in older larvae (2). The behavioral switch may be an adaptation that occurs as older larvae leave their food source—and thus darkness—to search for a suitable population site in the light. On page 499 of this issue, Gong *et al.* (3) identify two pairs of neurons in the *Drosophila* larval brain that control behavioral switch.

Conceptually, a behavioral switch can be implemented in different ways. For instance, two independent neuronal pathways could control behavioral extremes (attraction or repulsion), with the strength of each pathway determining the final outcome. Alternatively, a single neuron or pathway could switch between two different outputs depending on the sensory inputs received, on previous experience, or on intrinsic factors. Separate processing in two distinct pathways occurs in the adult fly's innate response to vinegar (1). In the olfactory system, sensory neurons express different odorant receptors and project to different glomeruli in the antennal lobe (4, 5), which pass information to higher brain centers. Vinegar odor activates several glomeruli, two of which are necessary and sufficient for attraction to low vinegar concentrations. At higher concentrations, an additional glomerulus is recruited, which changes the behavioral response to repulsion (1). Both responses are mediated by the same sensory neuron, but the mechanism that drives the behavioral switch has yet to be identified.

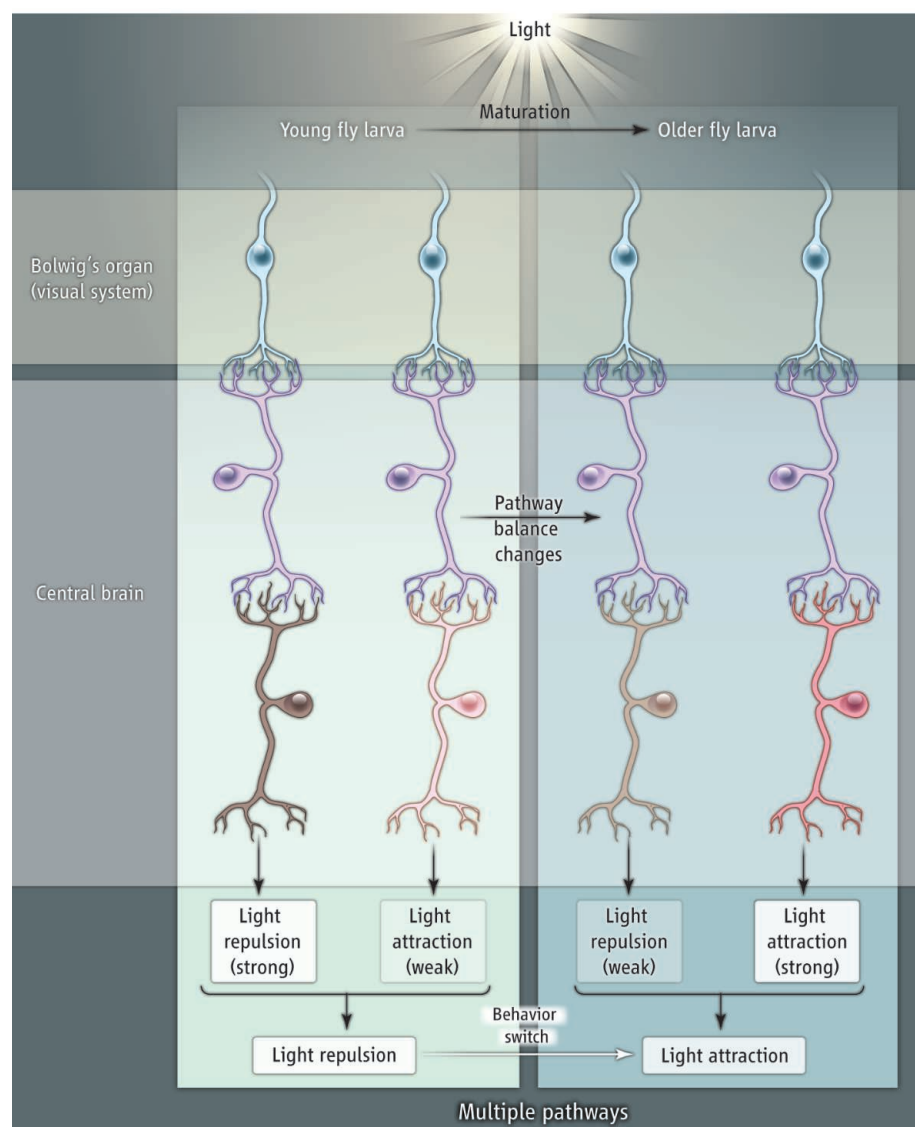
How do older *Drosophila* larvae switch from light avoidance to light preference? Gong *et al.* have identified a pair of neurons called NP394 in each central brain hemisphere that are required to maintain light avoidance in young larvae. When the function of these neurons was inhibited, either

by interfering with synaptic transmission or through hyperpolarization, even young larvae were attracted to light. By contrast, continuous hyperactivation of these neurons caused older larvae to avoid light instead of moving toward it. Thus, the activity of these neurons appears necessary and sufficient for controlling larval behavior in response to light.

The NP394 neurons most likely receive signals from ventral lateral neurons in the central brain through direct connections (synapses). The latter neurons develop into the master pacemaker that controls circadian rhythms in adult flies, but also have been

A specific neuronal circuit controls how flies respond to light during development.

implicated in controlling larval light avoidance (6). Ventral lateral neurons are innervated by photoreceptors of the Bolwig's organs, the visual system of the fly larva (7). When ventral lateral neurons were ablated in young larvae, the time course of light-induced Ca^{2+} influx into NP394 neurons changed, thus demonstrating a functional connection. Surprisingly, this ablation resulted in faster and slightly stronger Ca^{2+} influx into NP394 neurons upon light stimulation. This suggests that ventral lateral neurons block NP394 neuron function and that light releases this block. It also indicates that ventral lateral neurons



Behavior circuits. The circuit shown depicts how parallel neuronal pathways may control the innate behavioral response of an animal (fly larva) to a stimulus (light).

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are not the only light information input to NP394 neurons. This is consistent with the morphology of an NP394 neuron, which exhibits two postsynaptic arborizations—one close to the terminals of ventral lateral neurons and another close to midline neurons of the central nervous system.

The mechanism by which NP394 neurons control the behavioral switch from light avoidance to preference remains unclear. It is unlikely that these neurons change signaling properties over time because their hyperactivation in older larvae still results in avoidance. Perhaps NP394 neurons participate in aversive signaling, whereas a parallel neuronal circuit signals attraction (see the figure). In this scenario, NP394 neurons would become less active during development, thus reducing light avoidance. This agrees with current data, but further studies are needed to characterize NP394 function. For instance, it is important to determine if their activity decreases in older larvae. If this reduction is

due to decreased input from ventral lateral neurons, then Ca^{2+} imaging of NP394 neurons in older larvae would be informative. However, if it is the output of NP394 neurons that decreases with age, it will be necessary to perform Ca^{2+} imaging in downstream neurons, which have not yet been identified.

Another question regards the cues that change the output of the NP394 circuit during development. An intrinsic developmental timer could measure the age of the larva and change the properties of the neuronal circuit accordingly. Alternatively, the metabolic state of the animal could indicate when it is time to stop feeding (in the dark) and to enter pupation (in the light). This change could be linked to the developmental pathway controlled by the hormone ecdysone. Ecdysone triggers larval wandering and entry into pupation, and may affect the activity of NP394 neurons (8, 9). In *Drosophila*, ecdysone controls another dramatic sensory switch: In the adult Bolwig's organ, green-

sensitive photoreceptors die, while blue sensitive photoreceptors form an extraocular structure (the eyelet), but only after expression of blue opsin is turned off, and green opsin expression is turned on (10).

The finding of Gong *et al.* advances our understanding of how the animal brain interprets visual cues. It is also a step toward determining the neural basis of how both environmental and intrinsic cues modify innate behaviors.

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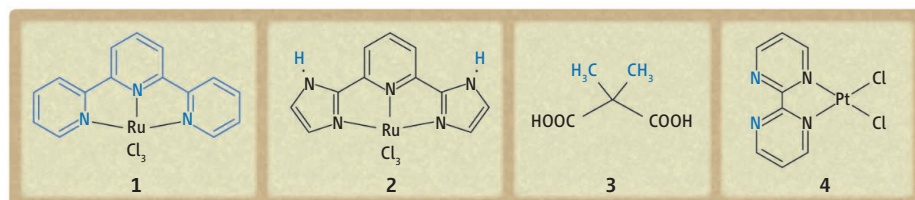
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CHEMISTRY

Creating Ligands with Multiple Personalities

Robert H. Crabtree

Ligands, once mere spectators in reactions catalyzed by transition metal complexes, are now being designed with additional functions that can augment the reactivity of the metal center. Classical ligands, such as the terpyridine in compound **1** (see the figure), often simply help to fill the coordination sphere of the metal and remain unchanged throughout a cycle of reactions. Even ligands that exert inductive effects—donating or withdrawing electrons to or from the metal's orbitals—can be tuned only by synthesizing a modified ligand. Multifunctional ligands that respond to effects such as changes in pH are now being developed, as illustrated by recent work by Hashiguchi *et al.* (1). They describe a ruthenium (Ru) complex **2** in which the ligand has a pair of labile protons (depicted in blue in the figure). The ligand is neutral but can lose one or two protons in strongly basic solution. This gain of one or two negative charges activates the Ru atom for carbon-hydrogen (C–H) bond reactions. In effect, three very different ligands



Switching from acid to base. The ligands on a metal center, such as terpyridine (shown in blue in **1**), usually remain unchanged during a catalytic reaction cycle. The new complex **2** reported by Hashiguchi *et al.* contains two labile protons, shown in blue, that are lost in the strongly basic medium. Compound **3** is one of the substrates successfully activated by complex **2** for hydrogen-deuterium exchange at 160°C. In Periana's older complex **4**, two nitrogens, in blue, are protonated in the highly acidic medium used for the catalytic reaction that converts C–H bonds to C–OSO₃H.

can be generated from a single core structure simply by changing pH.

The activation of normally unreactive C–H bonds (2) in complex molecules is one of the grand challenges of chemistry. The problem consists of converting a specific C–H bond into a C–X bond (where X is some functional group), typically with a metal complex as catalyst. Normally, such reactions are run under acidic conditions in order to promote binding of the C–H bonds to the metal center. In the initial step of the reaction, this interaction is weak, and the C–H bond of the substrate is

easily displaced by other neutral molecules in solution (including the solvent). The acid helps by protonating all potential competitors for the metal center (including the solvent), whereas the C–H bond will remain unprotonated even in strong acids.

Periana and co-workers previously described the successful exploration of strongly acidic conditions for C–H activation (3, 4) in which the metal (typically platinum) withdraws electrons from the C–H bond. In their more recent studies, the focus is on the other end of the pH scale and strongly basic

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conditions. The normal expectation is that this change would prevent easy binding of a C–H bond in the first step of C–H activation by failing to eliminate metal binding of competitive substances. In fact, a strong base accelerates the C–H activation rather than inhibiting it.

This unexpected result is achieved because the base affects the ligand rather than the metal center. The ligand undergoes double deprotonation under strongly basic conditions, so that the metal fragment in the complex now becomes an exceptionally strong electron donor to the metal. The C–H bond, although itself a weak electron acceptor, binds better under these conditions than it would in acid. Apparently, the electron-rich metal avoids binding the competitive substances present in the medium and prefers to donate electrons to the C–H bond. Surprisingly, both extremes, acid and base, are suitable for binding the C–H bond. Acidic conditions rely on the C–H bond as an electron donor. This second binding mode

described by Hashiguchi *et al.* relies on a situation that is much less often exploited, with the C–H bond acting as an electron acceptor. The bond is weakened by populating its antibonding orbitals.

The binding itself cannot be directly detected. Instead, Hashiguchi *et al.* detect it by running the reaction in a deuterated solvent; under these conditions, protons in the C–H bond are replaced by deuterium, but only in the presence of the metal compound. These results demonstrate that the interaction between the C–H bond and the metal indeed takes place. One example of a reactive substrate is shown as compound 3 in the figure, where the C–H bonds are labeled in blue. This work only considers the first step of C–H activation. The subsequent introduction of the X group remains for future studies.

These results are far from the only cases where ligands undergo modification during reaction. In previous work by Periana and co-workers (4), in strongly acidic condi-

tions, protonation of a ligand (complex 4 in the figure) reduced the electron density on the ligand and the platinum center. In other recent cases (5), ligands have taken up an electron and their properties have again been modified in the process. In yet another case (6), a ligand has been equipped with molecular recognition groups in order to bind a substrate in a specific mode and direct C–H activation to a precise point on the substrate. This area is still at a very early stage of development. We can anticipate many more interesting examples of ligands that do more than simply bind to metal.

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STRUCTURAL BIOLOGY

The Flu's Proton Escort

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The influenza A virus, which causes seasonal flu, poses a major threat to human health. One recent focus of research has been the M2 protein, a small membrane protein that enables hydrogen ions to enter the viral particle (1, 2); this “proton channel” plays a critical role in enabling the virus to infect cells and replicate and in other processes (3, 4). In recent flu seasons, a mutation in the M2 protein has rendered the virus resistant to two common antiviral drugs, amantadine and rimantadine. Efforts to develop new antiviral drugs would benefit from a better understanding of M2's structure and how drugs act on the protein, but recent studies have often produced conflicting results. This trend continues with two papers in this issue, by Sharma *et al.* (5) on page 509, and Hu *et al.* (6) on page 505. There are, however, possible explanations for the apparent inconsistencies in these and other recently reported structures (7, 8).

The M2 proton channel mediates the acidification of the interior of endosomes, intracellular compartments created by host cells.

The pH change enables viral RNA to escape into the cell and replicate. M2 has a 40-residue region that interacts with membranes (9) consisting of a transmembrane helix (which mediates tetramerization, drug-binding, and channel activity), followed by a basic amphiphilic helix important for budding of the virus from cellular membranes and release (scission) (4, 10). Research on M2 has been marked by debate since 2008, when investigators reported structures with anti-flu drugs bound at entirely different sites (11, 12). Even as this debate is settling in favor of a pharmacologically relevant site in the pore (13, 14), new papers (7, 8), including the two in this issue, stoke new controversy concerning the mechanism of proton conduction, and the structure of the cytoplasmic helix. Sharma *et al.* combine oriented solid-state nuclear magnetic resonance (SSNMR) measurements with previous electron paramagnetic resonance (EPR) measurements (15) to determine a backbone structure of the 40 residue region in phospholipid bilayers, resulting in a structure that differs significantly from recent solution NMR structures (7, 12). Hu *et al.* use magic angle spinning SSNMR to specifically probe the His residues that mediate proton translocation. Their high-resolution data are in good agreement with other recently published structures

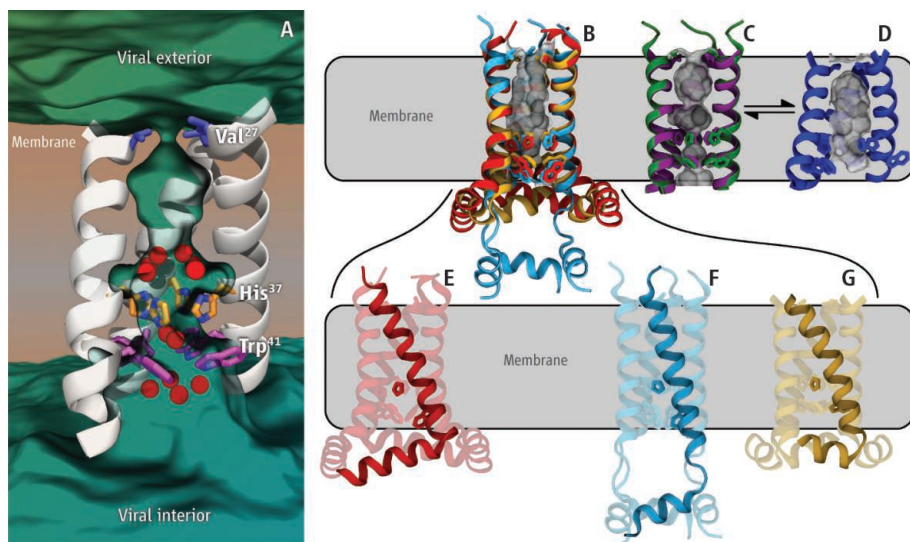
A flurry of structural data provides sometimes conflicting insights into the M2 proton channel.

(7, 8, 12, 16) but contradict many conclusions of Sharma *et al.* Here we offer explanations for the apparent paradox and relate the structures to the mechanism of proton transduction and vesicle budding/scission.

The EPR/SSNMR structure places the cytoplasmic helix at the C-terminal end of the bundle near the headgroup region of the bilayer (see the figure). By contrast, in the solution structure (12) the cytoplasmic helix forms a tetrameric bundle extending into the cytoplasm beyond the end of the TM domain (see the figure). The resolution of the solution structure is greater than that of the EPR/SSNMR, which was computed without the long-range distance and side-chain dihedral restraints used in computing previous solution and SSNMR structures (12, 16). Nevertheless, the structural differences appear larger than can be attributed to coordinate error and represent different conformational forms.

It would be premature to dismiss either as irrelevant, given that M2 is a highly dynamic protein with manifold conformations and functions (3, 4). Nevertheless, the EPR/SSNMR structure is more consistent with M2's ability to promote membrane budding and scission. M2 localizes to the neck of a budding vesicle, a region of extreme curvature that topologically resembles a donut-

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Flu in detail. The high-resolution crystal structure (8) of M2TM (A) is similar to spectroscopic structures of longer (B) constructs solved by SSNMR (5), (red), solution NMR (12) (light blue) or EPR (15) (yellow). The high-resolution structure (A) at intermediate pH shows a proton-conduction path consisting of layers of water molecules interleaved between the pore-lining side chains. The His³⁷ residues polarize intervening water molecules, encouraging entry of protons from the exterior. Water molecules are well positioned to increase the basicity of the N_ε atoms of His³⁷ by simultaneously forming hydrogen bonds to the carbonyl of Gly²⁴. Interactions between N_ε and the electron-rich face of Trp⁴¹ further stabilize the His-box. In the doubly charged state, this interaction is mediated by an intervening dimer of water molecules, which are weakened or converted to direct side-chain-side-chain interactions (8) in the neutral state. Additional water clusters toward the interior provide indirect charge stabilization and complete an exit pathway. (C) Low protonation (PDB ID codes: 2KQT, green; 3LBW, purple) (11, 16) versus (D) greater protonation (PDB ID code 3BKD, blue) (8) show that increasing protonation of the His box/water cluster causes the pore to contract on the exterior and expand near the interior, promoting movement of protons past the Trp⁴¹ gate into the interior of the virus (11). The structure of the C-terminal amphiphilic helix of Sharma *et al.* seen in (E) differs markedly from that of Schnell and Chou (12) in (F) but resembles the EPR model of Nguyen, Howard, and co-workers (G) (15).

hole. Such saddle-shaped surfaces have negative Gaussian curvature characterized by orthogonally directed negative and positive local curvature. The EPR/SSNMR structure positions the M2 cytoplasmic helix appropriately to promote negative Gaussian curvature by interacting with phospholipid headgroups (17). Future questions will likely focus on how the TM domain modulates the cytoplasmic helix, which has considerable activity as an isolated peptide.

The highest resolution studies of the channel-forming pore of M2 were determined using the TM peptide (M2TM), which is sufficient for proton channel formation. Electrophysiological measurements of a similar TM construct expressed in *Xenopus* frog oocytes showed channel activity within a factor of two of the full-length protein (18). To determine whether the cytoplasmic peptide interactions seen in the EPR/SSNMR and solution NMR structures are important for proton channel activity, Rossman *et al.* simultaneously replaced all five hydrophobic residues along one face of the cytoplasmic helix (F47, F48, I51, Y52, F55) with Ala in the full-length protein (4, 10). As expected, the surface expression level, proton flux, pH-activation, and

drug-binding of this quintuple mutant were indistinguishable from wild type, while it had greatly diminished scission activity. Thus, although the detailed interactions of the cytoplasmic helix seen in the EPR/SSNMR structure might relate to scission they do not influence channel activity.

The structures of M2TM (8, 11, 16,) and M2TM+cytoplasmic constructs (15, 12) together with papers in this issue, suggest a unified mechanism for proton conduction (see the figure). Protons enter the channel through a narrow opening, the Val²⁷-valve into an aqueous pore leading to His³⁷. Four His³⁷ and Trp⁴¹ side-chains associate in an interaction that also inhibits reverse flow of protons out of an acidified virus. The His³⁷ tetrad is primed for conduction by binding two protons with high affinity (a negative log of dissociation constant or pK, value of ~8) (19). The affinity (pK~6) for the third proton appears evolutionarily tuned to match the pH of an acidifying endosome (20). In the shuttle mechanism (21), binding of the third proton from the exterior induces loss of a proton to the internal side, regenerating the +2 state.

The high-resolution crystal structure suggests that incoming protons are statistically

delocalized in an imidazole/water cluster (8) (see the figure) with some parallels to the storage of electrons in bio-inorganic clusters. Addition of the third proton increases the hydration of His³⁷ (6), and induces dynamics on a rate more rapid than that of proton flux (6), thereby promoting proton release into the viral interior. Finally, computation (22) and the experimental structures suggest that as the His³⁷ tetrad is increasingly protonated, the cytoplasmic end of the bundle dilates and the exterior-facing Val²⁷-valve constricts. This transporter-like mechanism also explains the greater rate of conductance when the pH is low outside but high inside, versus the opposite situation. So long as the His residues are deprotonated by high pH outside they remain kinetically protected by Trp⁴¹ from the low pH inside. This mechanism is also consistent with the low-level counter-flow of alkali metal cations, which accompanies proton flux in sealed vesicles to maintain electrical neutrality (23).

While these fundamental steps in conduction are becoming clear, significant questions remain. For example, in several structures, the four His³⁷ residues form a square His-box (see the figure); the imidazole side chains are connected via hydrogen-bonds to intervening water molecules also seen in Hu *et al.*'s SSNMR studies. By contrast, Sharma *et al.*, propose a different geometry that is inconsistent with Hu *et al.*'s direct measurements of side-chain geometry at both low and high pH. However, their structural data are limited to backbone orientational restraints, which do not match the resolution of a 1.65 Å x-ray structure (8) or direct side-chain distance and dynamic measurements (6).

Other remaining mechanistic questions include: whether the large-scale transporter-like backbone motions accompany the transport of each proton through M2 (which is consistent with its transporter-like maximal turnover of about 100 protons/s), and whether a permeating proton is shuttled on and off an individual His³⁷, or rapidly delocalized throughout the water-cluster/His-box (8). It has also been proposed that a permeating proton might bypass His³⁷ altogether and travel via a chain of water molecules (24). Clearly, biophysicists will remain busy resolving these issues, illuminating interactions that can be mined for the design of new generations of anti-flu medications.

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OCEANS

Chaos in the Gulf

Jean-Luc Thiffeault

The magnitude of the oil spill into the Gulf of Mexico after the Deepwater Horizon disaster has been estimated at more than 4.4 million barrels (1). The subsequent environmental impact will rely both on monitoring and on the ability to accurately model the dispersal of the oil. On page 486 of this issue, Mezić *et al.* (2) present a modeling study that describes how the oil dispersed and attempts to predict how the spill will evolve. The mechanism of dispersal is complex, a question of transport and mixing, along with a dose of evaporation and chemistry. Mixing forms an entire branch of study, one that is closely linked to dynamical systems—the mathematical study of time-evolving solutions of differential equations. In the context of fluid mechanics, this link was first recognized by Arnold (3), Henon (4), and later by Aref (5), and it essentially boils down to chaos.

Chaos is associated in the popular imagination with the so-called butterfly effect, where initially small disturbances can grow exponentially to overwhelm any precision we may have used in computing trajectories of our dynamical system, as discovered by Lorenz (6). Take a container filled half with red fluid, half with blue. If the motion of small parcels of fluid is chaotic, then two blue par-

cels that were initially neighbors find themselves distantly separated after some time and are mixed in with red parcels. It is the chaotic motion of fluid parcels that rapidly leads to an uncorrelated or mixed state.

If the flow is not chaotic everywhere then things can get more complicated. Some regions of the flow may mix well, others may not. Jupiter's Great Red Spot is a famous example of a segregated flow region that mixes very little with its surroundings. One of the challenges in the dynamical systems approach to mixing is to predict which regions involve flows that will lead to good mixing, and which ones don't.

Since the 1980s, better analytical tools have been developed, spurred partly by improvements in computing power. The workhorse of chaotic dynamics is the Lyapunov exponent, a quantity or metric that provides a measure of the rate of separation of neighboring trajectories. Mathematical theorems regarding Lyapunov exponents assume either a periodic system or one that can be allowed to run for arbitrarily long times. Clearly, this is not well suited to experimental observations, which are almost never periodic and certainly never infinite. Other types of exponents were therefore introduced, for example, finite-time and finite-size Lyapunov exponents. What they all share in common is that their value depends on exactly when and where a measurement is taken. This is a feature of the modeling as

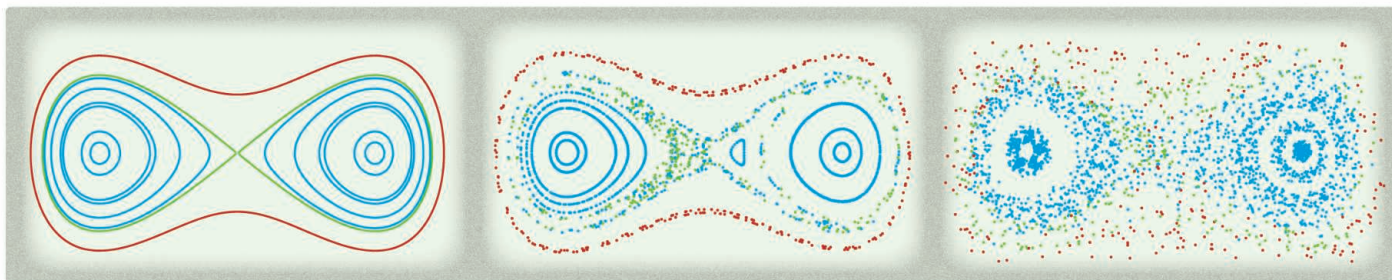
Sophisticated mathematical analysis is providing insights into how the oil released in the Deepwater Horizon disaster is dispersing.

the local extrema or ridges that develop in the contour plot of finite-time Lyapunov exponents help identify barriers to transport (7–9). These transport barriers are such that fluid finds it difficult to cross them; for example, the Gulf Stream is a well-known transport barrier that bisects the Atlantic Ocean.

But transport barriers can be difficult to identify, hence the need for a systematic mathematical description. The ridge structure has been called the skeleton of a flow. The skeleton of transport barriers distills simple, accessible information from the overwhelming complexity contained in fluid trajectories.

Once transport barriers are located, we can predict where oil may or may not end up. Locating transport barriers becomes more difficult as a flow's complexity is increased (see the figure). The left frame shows a steady flow, where every orbit is closed: The concept of a transport barrier is not useful here, but note that the red orbits are outside the two eyes, the blue orbits are inside, and the green orbits divide the two regions. The middle frame shows the same system affected by a small time-periodic perturbation. The barrier between inside and outside is smeared, but it is still easy to make out visually. In the right frame, the perturbation is random in time: Clearly, there are still orbits trapped inside, but it becomes harder to characterize the boundaries of the interior regions. In real oceanic data this task is even harder.

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In the mix. (Left) Orbits of a nonchaotic dynamical system. (Middle) When the system is influenced by a time-periodic perturbation, some orbits exhibit chaotic behavior and escape from the two eyes, as seen in this stroboscopic map taken

at every period. (Right) When the perturbation is nonperiodic, as considered by Mezić *et al.* (2), a stroboscopic map is less revealing, but it is still apparent that there are orbits inside the two eyes that never escape.

Mezić *et al.* aim to find not just barriers but also regions that are particularly active in mixing the fluid. Such regions can lead, for instance, to a more rapid breakdown of oil into less harmful microdroplets. Their models rely on the concept of hyperbolicity of a flow: Hyperbolic flows are good at stretching fluid parcels. As the flow in the Gulf of Mexico is not periodic, and we have limited data, they introduce mesohyperbolicity: hyperbolicity on average. This reflects the fact that a typical fluid parcel may meet regions that are both favorable

and adverse to mixing over a finite stretch of time. Mesohyperbolicity is a generalization to finite time of the concept of a Floquet analysis for periodic flows, where we can deduce the long-time behavior by examining a single period.

Mezić *et al.* were successful in describing how the oil was dispersed after the Deepwater Horizon oil leak. The oil spill occurred just as interest in these dynamical systems ideas is the subject of renewed interest. But hopefully we will be better prepared when another oil leak occurs, and applied mathematicians can

work in conjunction with cleanup crews to predict where efforts are best focused.

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EVOLUTION

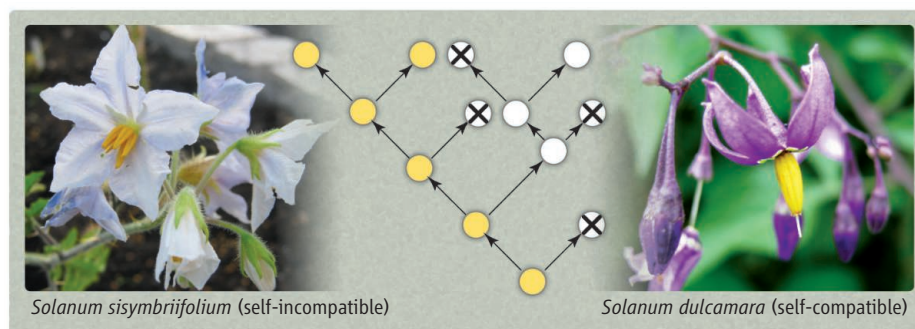
The Long-Term Benefits of Self-Rejection

Stephen I. Wright and Spencer C. H. Barrett

Charles Darwin provided the key explanation for the striking adaptive fit of organisms to their environments; traits that confer a greater chance of survival and reproduction spread through populations by the process of evolution by natural selection. But natural selection has no foresight; only immediate advantages matter. So the same trait that confers higher fitness in the short term could also increase the chance that a species eventually becomes extinct, or decrease the chance that it will form new species. Conversely, another trait may enhance a species' ability to diversify, leading to more species with that trait.

Many evolutionary biologists refer to such trait-specific effects on species diversification as "species selection," an evolutionary process that occurs above the species level (1). Biologists mostly accept the concept, but its general importance has not been clear. On page 493 of this issue, however, Goldberg *et al.* (2) provide a compelling case for the importance of species selection. In a study of hermaphroditic plants, in which individuals function as both males and females, they show that one trait—the ability of an individual to prevent self-fertilization by recognizing and rejecting its own pollen—appears to have strongly affected species diversification in the nightshade family, Solanaceae.

Hermaphroditic plants exhibit either self-compatibility (SC), which means an individ-



Know thyself. Species selection maintains self-incompatibility. Self-incompatible species (yellow circles) frequently experience evolutionary transitions to self-compatibility (white circles), leading to the potential loss of the trait over evolutionary time. This is balanced by a higher rate of extinction of self-compatible species (crosses), causing the stable maintenance of both types over long periods of evolutionary time.

ual can act as both mother and father to its own offspring, or self-incompatibility (SI), which enforces outcrossing. SI is a genetically controlled physiological mechanism that is mediated by protein-protein interactions. It is analogous to a lock-and-key mechanism, in which the maternal parent recognizes pollen grains (or pollen tubes) that share alleles, initiating a biochemical pathway that rapidly blocks fertilization (3). The primary advantage of SI is that it avoids the harmful effects of inbreeding, particularly self-fertilization. However, SI comes at a cost because plants produce offspring that are just 50% related to them, rather than 100%, thus reducing transmission of their own genes to the next generation. Also, fertility can be severely compromised if pollinators or mates are unavailable. Because of these disadvantages, flowering plants have lost SI on numerous occasions during their evolutionary history, resulting

in SC. Because of the complexity of SI, the trait is rarely, if ever, regained. This implies that SI may be on its way to extinction, yet at least half of all living flowering plant species are SI. Perhaps SI has some macroevolutionary advantage allowing it to be maintained by species selection.

Evaluating the importance of species selection is difficult, because it is tricky to estimate rates of character transition (such as a switch from SI to SC) separately from rates of speciation and extinction. Recent statistical advances, however, now allow researchers to analyze this question by using evolutionary trees (4, 5). Using extensive phylogenetic information on species relationships in Solanaceae (which includes important crop plants, such as tomato, potato, and tobacco), the authors reconstructed the evolutionary history of transitions from SI to SC. They relied on well-resolved clades (groups of species

that share common ancestry). In this family, the evolutionary transition from SI to SC has occurred independently on numerous occasions. This is important because it improves statistical power and increases confidence that the transition itself is closely associated with differences in species diversification.

Goldberg *et al.* showed that SI lineages have a higher net diversification rate—a key quantity that determines the rate of increase in species numbers—than SC lineages. One key finding of their study is that this difference is not due to a higher rate of speciation for SI lineages; in fact, inferred speciation rates were higher in SC lineages. Instead, SC lineages have higher extinction rates than SI lineages. As a result, SI lineages have higher net diversification rates that apparently have been sufficient to counter-balance the repeated loss of the trait, allowing it to be maintained over evolutionary time (see the figure). The results provide a convincing macroevolutionary explanation for how SI has persisted over tens of millions of years despite its repeated breakdown to SC.

Given these results, a number of outstanding questions remain. Most importantly, why should SC plants that have the potential to self-fertilize experience higher rates of extinction? This study did not address the issue of how much “selfing” occurs in SC species, but the process is commonly associated with reduced

genetic diversity and lower rates of recombination. This reduces the chance of eliminating deleterious mutations and can decrease opportunities for adaptive mutations to succeed (6), both of which can increase the probability of extinction. Because some of the species included in the study probably self-fertilize at high rates, it is possible that the actual driver of differential diversification is not SI *per se* but the rate of self-fertilization. If the researchers had been able to use actual data on rates of cross- and self-fertilization (rather than only classifying plants as SC or SI), even stronger differences in diversification may have been found. However, obtaining this information for the many species included in this study would be a Herculean task.

Different approaches to studying the evolutionary consequences of selfing have provided conflicting results. Molecular work on protein evolution has found little evidence that selfing populations accumulate harmful mutations (7, 8). In contrast, phylogenetic studies indicate that selfing species commonly produce short branches on evolutionary trees and appear to be more prone to extinction (9, 10). One possible explanation is that molecular studies have focused on too coarse a level to detect the predicted differences in the efficacy of selection. New approaches that enable simultaneous estimates of the strength of positive and negative selection are likely to be

more powerful (11, 12). Also, with few exceptions (8), molecular evolutionary studies have not been done with a large number of species, making it difficult to detect repeated declines in fitness of selfing lineages. Finally, SC lineages may experience higher extinction rates for reasons unrelated to mutational decay. For example, SI species often occur in relatively large, often long-lived, populations, and these demographic properties may make them more likely to persist over longer time scales. The causes of differences in diversification rates among lineages remain a central issue in evolutionary biology, but this illuminating study indicates that we should not ignore macroevolutionary processes in trying to understand the maintenance of adaptations and biodiversity.

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EPIDEMIOLOGY

Environment and Disease Risks

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Although the risks of developing chronic diseases are attributed to both genetic and environmental factors, 70 to 90% of disease risks are probably due to differences in environments (1–3). Yet, epidemiologists increasingly use genome-wide association studies (GWAS) to investigate diseases, while relying on questionnaires to characterize “environmental exposures.” This is because GWAS represent the only approach for exploring the totality of any risk factor (genes, in this case) associated with disease prevalence. Moreover, the value of costly genetic information is diminished when inaccurate and imprecise environmental data lead to biased inferences regarding gene-environment interactions (4). A more comprehensive and quantitative view of environmental expo-

sure is needed if epidemiologists are to discover the major causes of chronic diseases.

An obstacle to identifying the most important environmental exposures is the fragmentation of epidemiological research along lines defined by different factors. When epidemiologists investigate environmental risks, they tend to concentrate on a particular category of exposures involving air and water pollution, occupation, diet and obesity, stress and behavior, or types of infection. This slicing of the disease pie along parochial lines leads to scientific separation and confuses the definition of “environmental exposures.” In fact, all of these exposure categories can contribute to chronic diseases and should be investigated collectively rather than separately.

To develop a more cohesive view of environmental exposure, it is important to recognize that toxic effects are mediated through

A new paradigm is needed to assess how a lifetime of exposure to environmental factors affects the risk of developing chronic diseases.

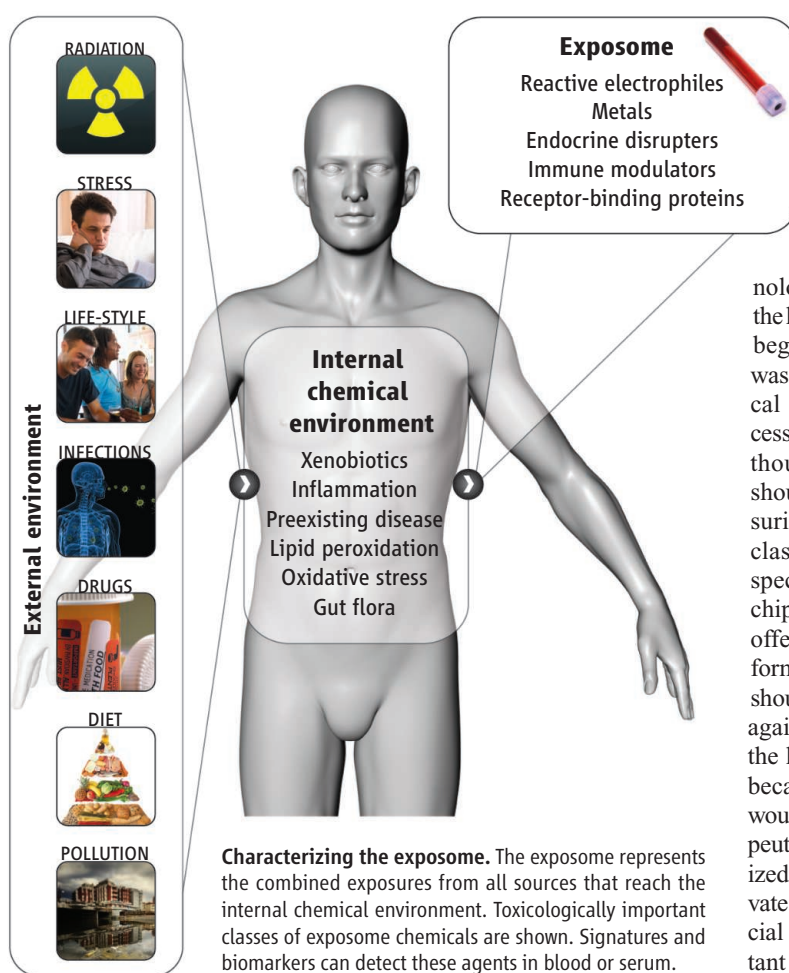
chemicals that alter critical molecules, cells, and physiological processes inside the body. Thus, it would be reasonable to consider the “environment” as the body’s internal chemical environment and “exposures” as the amounts of biologically active chemicals in this internal environment. Under this view, exposures are not restricted to chemicals (toxicants) entering the body from air, water, or food, for example, but also include chemicals produced by inflammation, oxidative stress, lipid peroxidation, infections, gut flora, and other natural processes (5, 6) (see the figure). This internal chemical environment continually fluctuates during life due to changes in external and internal sources, aging, infections, life-style, stress, psychosocial factors, and preexisting diseases.

The term “exposome” refers to the totality of environmental exposures from conception onwards, and has been proposed to be a

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critical entity for disease etiology (7). Recent discussion has focused on whether and how to implement this vision (8). Although fully characterizing human exposomes is daunting, strategies can be developed for getting “snapshots” of critical portions of a person’s exposome during different stages of life. At one extreme is a “bottom-up” strategy in which all chemicals in each external source of a subject’s exposome are measured at each time point. Although this approach would have the advantage of relating important exposures to the air, water, or diet, it would require enormous effort and would miss essential components of the internal chemical environment due to such factors as gender, obesity, inflammation, and stress. By contrast, a “top-down” strategy would measure all chemicals (or products of their downstream processing or effects, so-called read-outs or signatures) in a subject’s blood. This would require only a single blood specimen at each time point and would relate directly to the person’s internal chemical environment. Once important exposures have been identified in blood samples, additional testing could determine their sources and methods to reduce them.

To make the top-down approach feasible, the exposome would comprise a profile of the most prominent classes of toxicants that are known to cause disease, namely, reactive electrophiles, endocrine (hormone) disruptors, modulators of immune responses, agents that bind to cellular receptors, and metals. Exposures to these agents can be monitored in the blood either by direct measurement or by looking for their effects on physiological processes (such as metabolism). These processes generate products that serve as signatures and biomarkers in the blood. For example, reactive electrophiles, which constitute the largest class of toxic chemicals (6), cannot generally be measured in the blood. However, metabolites of electrophiles are detectable in serum (9), and products of their reactions with blood nucleophiles, like serum albumin, offer possible signatures (10). Estrogenic activity could be used to monitor the effect of endocrine dis-



Characterizing the exposome. The exposome represents the combined exposures from all sources that reach the internal chemical environment. Toxicologically important classes of exposome chemicals are shown. Signatures and biomarkers can detect these agents in blood or serum.

ruptors and can be measured through serum biomarkers. Immune modulators trigger the production of cytokines and chemokines that also can be measured in serum. Chemicals that bind to cellular receptors stimulate the production of serum biomarkers that can be detected with high-throughput screens (11). Metals are readily measured in blood (12), as are hormones, antibodies to pathogens, and proteins released by cells in response to stress. The accumulation of biologically important exposures may also be detected as changes to lymphocyte gene expression or in chemical modifications of DNA (such as methylation) (13).

The environmental equivalent of a GWAS is possible when signatures and biomarkers of the exposome are characterized in humans with known health outcomes. Indeed, a relevant prototype for such a study examined associations between type 2 diabetes and 266 candidate chemicals measured in blood or urine (14). It determined that exposure to certain chemicals produced strong associations with the risk of type 2 diabetes, with effect sizes comparable to the strongest genetic loci reported in GWAS. In another study, chromo-

some (telomere) length in peripheral blood mononuclear cells responded to chronic psychological stress, possibly mediated by the production of reactive oxygen species (15).

Characterizing the exposome represents a technological challenge like that of the human genome project, which began when DNA sequencing was in its infancy (16). Analytical systems are needed to process small amounts of blood from thousands of subjects. Assays should be multiplexed for measuring many chemicals in each class of interest. Tandem mass spectrometry, gene and protein chips, and microfluidic systems offer the means to do this. Platforms for high-throughput assays should lead to economies of scale, again like those experienced by the human genome project. And because exposome technologies would provide feedback for therapeutic interventions and personalized medicine, they should motivate the development of commercial devices for screening important environmental exposures in blood samples.

With successful characterization of both exposomes and genomes, environmental and genetic determinants of chronic diseases can be united in high-resolution studies that examine gene-environment interactions. Such a union might even push the nature-versus-nurture debate toward resolution.

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Neutrino Spectroscopy Can Probe the Dark Matter Content in the Sun

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Dark-matter particles are known to accumulate inside large gravitational celestial bodies (1). Given that the Sun is the largest body closest to Earth, it is therefore an obvious place to look for dark-matter signatures [e.g., (2–4)]. If dark matter in the local halo consists of weakly interacting particles, gravitational capture by the Sun is inevitable, resulting in accumulation of dark matter within its core as these particles collide inelastically with baryons. Small changes result in the Sun's structure (5) that is sensitive to dark-matter properties (6), especially light dark matter (in the 4- to 20-GeV particle mass range). By using an updated solar standard model that shows a seismic diagnostic similar to those of other solar standard models (7–9), we computed a series of solar models evolving within dark-matter haloes, for which we have estimated the impact of dark-matter particles in the present-day Sun [supporting online material (SOM)]. The nuclear reactions that produce electron neutrinos occur at different locations in the Sun, leading to specific distributions of various neutrino fluxes. Maximum neutrino production of the proton-proton (pp) chain occurs at 4%, 6%, 9%, and 10% of the solar radius for ^8B -v, ^7Be -v, pep-v, and pp-v, respectively. In the case of CNO cycle reactions, the maximum occurs at 5% of the solar radius for ^{15}O -v and ^{17}F -v. In the case of ^{13}N -v, two maxima occur at 5 and 6% at the solar radius. It is possible to use these distinctive dependences of neutrino production reactions in the solar interior to define a unique signature for the

existence of dark-matter particles in the Sun's core. According to our calculations, the presence of dark matter in the core can lead to neutrino flux variations as large as a 30% increase for ^8B -v and a 2% decrease for pp-v (Fig. 1, left), corresponding to a 4% decrease in the central temperature and a 3% increase in the central density of the Sun.

Identical results were independently obtained in the case of asymmetric dark matter (10). These solar temperature changes are difficult to probe with acoustic waves but can be measured by future solar neutrino experiments. A complementary but independent probe of the solar core can also be provided by future gravity wave seismology (11, 12).

Previous experiments (13, 14) have shown that the ^8B -v and ^7Be -v neutrino fluxes are in agreement with the values predicted by most of the solar standard models. By using the neutrino fluxes of the present solar standard model as a reference, we predicted the neutrino flux variations for a range of solar models evolving in haloes of dark matter in the cases of annihilating and nonannihilating low-mass particles (Fig. 1, right).

The variations can be as large as 90% in the case of nonannihilating particles. A complete quantitative account of the physical processes involved in the evolution of the Sun is fundamental for defining the Sun's internal structure (15).

Nevertheless, our results demonstrate that the possible existence of a dark-matter isothermal core in the Sun will produce a distinct signature for sev-

eral neutrino flux measurements that will help distinguish the dark-matter modulation of the solar core from other possible astrophysical sources.

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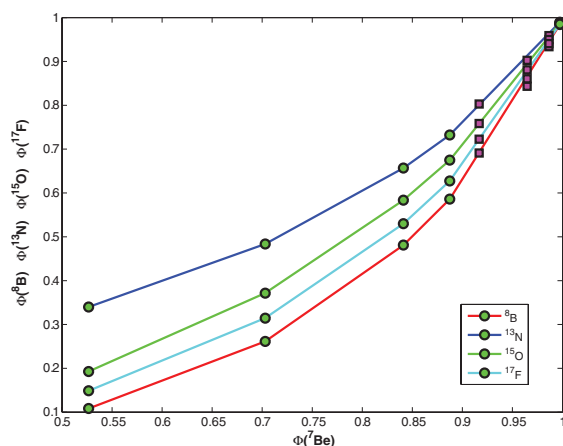
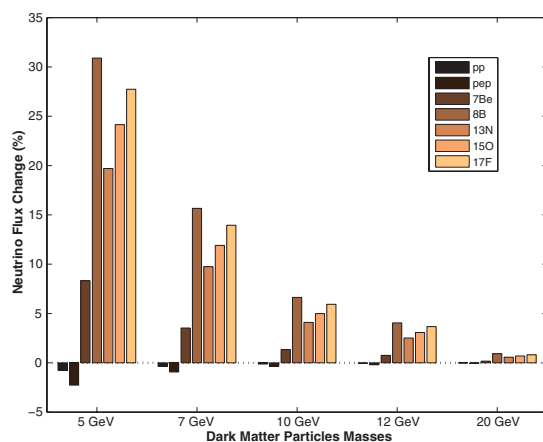
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Fig. 1. (Left) Percentage changes in solar neutrino fluxes for the pp chain (pp-v, pep-v, ^7Be -v, and ^8B -v) and CNO cycle (^{13}N -v, ^{15}O -v, and ^{17}F -v) in the case of weakly annihilating (spin-dependent) light dark matter. The neutrino flux percentage change of each species is compared to the current flux values as predicted by the solar standard model (7–9). **(Right)** Fractional changes in solar neutrino fluxes of ^8B -v (red curve), ^{13}N -v (dark blue curve), ^{15}O -v (green curve), and ^{17}F -v (light blue curve) as a function of ^7Be -v for different scenarios of dark-matter particles accumulating in the Sun's core: nonannihilating dark particles with masses 5, 7, 10, 12, and 50 GeV (green circles) and weakly annihilating (spin-dependent) dark particles with masses 5, 7, and 10



GeV (violet squares). The mass values increase from lower to higher values of ^7Be -v. The fluxes of each species were normalized to the current flux values as predicted by the solar standard model.

Detection of Water in the LCROSS Ejecta Plume

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Several remote observations have indicated that water ice may be presented in permanently shadowed craters of the Moon. The Lunar Crater Observation and Sensing Satellite (LCROSS) mission was designed to provide direct evidence (1). On 9 October 2009, a spent Centaur rocket struck the persistently shadowed region within the lunar south pole crater Cabeus, ejecting debris, dust, and vapor. This material was observed by a second “shepherding” spacecraft, which carried nine instruments, including cameras, spectrometers, and a radiometer. Near-infrared absorbance attributed to water vapor and ice and ultraviolet emissions attributable to hydroxyl radicals support the presence of water in the debris. The maximum total water vapor and water ice within the instrument field of view was 155 ± 12 kilograms. Given the estimated total excavated mass of regolith that reached sunlight, and hence was observable, the concentration of water ice in the regolith at the LCROSS impact site is estimated to be $5.6 \pm 2.9\%$ by mass. In addition to water, spectral bands of a number of other volatile compounds were observed, including light hydrocarbons, sulfur-bearing species, and carbon dioxide.

Neutron scattering measurements by Lunar Prospector (2) indicated increased concentrations of hydrogen at latitudes within 20° of the poles; subsequent observations from Earth and spacecraft have provided further evidence for the presence of trace amounts of ice or bound OH on the Moon (3–5). Neither the chemical form of the hydrogen-bearing compounds nor their origins has been determined; a variety of studies have postulated sources, including solar wind, asteroids, and comets (6–10). Clementine radar observations showed a bright radar return from the interior of Shackleton Crater consistent with volumetric scattering by water ice (11), a process requiring this ice to be relatively pure ($\sim 90\%$) and 1 to 2 m thick. The goal of the Lunar Crater Observation and Sensing Satellite (LCROSS) was to provide ground truth at one location—the crater Cabeus—for these observations by crashing a spent rocket stage into the Moon and observing the ejected debris, including any hydrogen species, from instruments on a trailing spacecraft.

The impactor was the spent upper stage of the Atlas V rocket, the Centaur, which propelled both the Lunar Reconnaissance Orbiter (LRO) and LCROSS to the Moon. Shortly after launch, LRO separated from the LCROSS-Centaur stack,

the Centaur vented its remaining fuel, and control was assumed by the LCROSS Shepherding Spacecraft (SSc). The LCROSS SSc then controlled the Centaur for the next 4 months, performing maneuvers to allow the sun to bake out residual water (12), perform instrument calibrations, and ultimately target the Centaur at the planned impact site. Approximately 9 hours before impact, the SSc separated from the Centaur, performed a braking burn to build a 4-min separation between itself and the Centaur at impact, and oriented its instruments in the direction of the targeted impact site. About 1 hour before impact, the nine LCROSS instruments were powered on and began taking data (13). Observations from the near-infrared (NIR) and ultraviolet/visible (UV/Vis) spectrometers are described here [(14) and supporting online material (SOM)].

The LCROSS target site was selected using a variety of criteria; the two most important were the altitude at which ejecta would be illuminated by sunlight and evidence of elevated levels of hydrogen from previous measurements. Topographic data and its orientation to the Sun indicated that Cabeus was the most compelling target (15–18). Substantial levels of hydrogen were indicated in Cabeus by both the Lunar Prospector Neutron Spectrometer (LPNS) and the Lunar Exploration Neutron Detector (LEND) (2, 18). The height required for the debris to reach sunlight was 833 m at the Centaur impact site and time, based on the comparatively low latitude of Cabeus ($\sim 81.5^\circ$ S) and a cleft in the crater rim topography on the sunward side at the time of impact. This observation geometry also provided a dark background for the nadir spectrometer measurements. The area selected for impact showed temperatures

less than 50 K (19), making it a likely cold trap for volatiles.

Immediately after the impact [see (20) for details], a hot (1000 K) vapor cloud emerged from the crater, followed by low- and high-angle (relative to the surface) ejecta. The two spectrometers measured both thermal emission from the hot (~ 300 to 1000 K) ejecta (derived from a fit of the NIR spectrometer spectra and consistent with LRO and Diviner Lunar Radiometer measurements) and solar scattering from sunlit debris (both NIR and UV/Vis spectrometers). The debris cloud reached sunlight about 1 s after impact, and peak brightness in UV/Vis spectra was approximately 17 s after impact. Brightness waned to a persistent level substantially above preimpact background for another 223 s thereafter (Fig. 1). The total radiance time profile from the NIR spectrometer was unlike that from a simply dissipating scattering dust source: Radiance jumped well above background just after impact, but the total radiance continued to rise during the next 180 s. The lower initial total radiance measured by the NIR spectrometer (one-fifth that of the UV/Vis radiance) is attributed to two factors. First, the ejecta cloud did not efficiently scatter NIR; models suggested a ratio of UV/Vis-to-NIR of 2.5, but the observed ratio was 5, indicating grains smaller than a few microns. Second, a high concentration of dust or species that absorbed

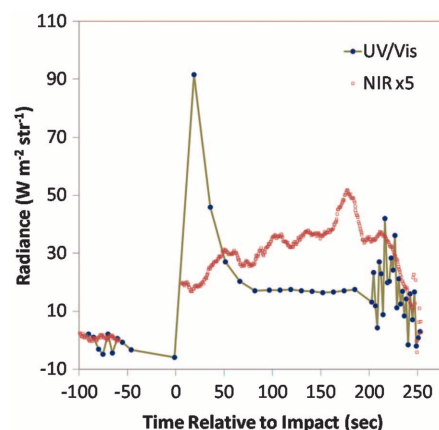


Fig. 1. The total ejecta cloud radiance (summed across sampled wavelength space) measured by the NIR and UV/Vis spectrometers as a function of time relative to impact. The total radiance for the ejecta cloud alone was derived by subtracting from the total measurement of radiance a fit to scattered light and light from illuminated surfaces in the instruments' FOVs. For clarity, the NIR radiance has been multiplied by a factor of 5. The gap in the NIR data ~ 1 min before impact corresponds to when the nadir NIR spectrometer was placed into “flash” mode, a mode in which only five spectral positions are sampled but are done so at about 72 Hz. These data are not used in the analysis presented here. Also, the reconfiguration of the UV/Vis spectrometer for long integration times results in a sampling pause just before impact.

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in the NIR (described below) lowered integrated radiance across most of the NIR spectrum, an effect also seen both experimentally and in the Deep Impact mission (21). After the impact, the

abundance of NIR absorbers diminished as the plume expanded kinetically and departed the field of view (FOV) of the instrument. However, in general, at wavelengths longer than about 1.9 μm ,

the radiance increased as range decreased between the instrument and the warm, emitting crater (Fig. 2).

The nadir NIR spectrometer measurements after impact were dynamic, with significant variations across the measured spectrum as a function of time (Fig. 2). We used 180 measurements taken in the ~ 90 s before impact to generate a reference spectrum against which postimpact spectra could be compared. After impact, various time series of scans were averaged to improve signal-to-noise ratios. Each NIR spectrometer scan took ~ 0.6 s; thus, averaging across 30 scans effectively averages over ~ 20 s. The averaging time period was guided by discernible periods of distinct NIR phenomenology, as indicated by the peaks and valleys in the total NIR radiance (Fig. 1). These differentiable periods are likely to be the result of changes in the FOV of the instrument (during descent toward the impact site) (22), and changes in the morphology of the ejecta and vapor clouds, as debris moved into sunlight and volatiles continued to sublimate from ejecta grains and the warmed surfaces in and around the impact crater. During the earliest periods after impact, there was a clear indication of a NIR contribution from scattered solar light (see Fig. 2, spectra for periods 0 to 24 and 24 to 30 s). This contribution is seen as an upturn at wavelengths shorter than ~ 1.5 μm . At longer wavelengths, the continuum is composed of a combination of weak scattering from ejecta debris and the surface, and emission from the hot crater (23). Over all spectral periods, the continuum generally appeared to be heavily eroded by a variety of absorbers. After about 30 s, gas absorption bands, which were

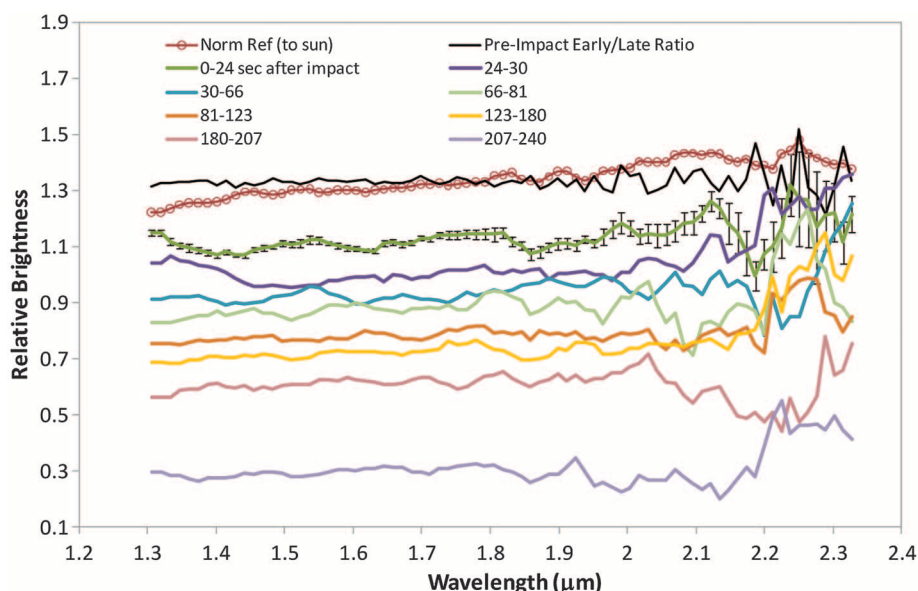


Fig. 2. Scaled reflectance NIR spectra for different time periods relative to impact, with each spectrum offset vertically in brightness for clarity. Each spectrum is an average over the time period indicated in the figure key (50 s is ~ 83 spectra) and is smoothed over 5 spectral bins (~ 0.07 μm). Also shown is a preimpact reference spectrum [divided by the solar radiance convolved to the instrument resolution (14) and normalized], constructed from an average of 180 scans taken ~ 30 min before impact, as well as the ratio of the first 90 of these scans (Pre-Impact Early) and the second 90 of these scans (Pre-Impact Late). The spectra measured after impact were divided by a preimpact reference spectrum that was generated from spectra taken during the last 90 s just before impact, to derive the relative reflectance spectra of the ejecta cloud. ± 1 SD error bars are shown for reference on one of the postimpact spectra.

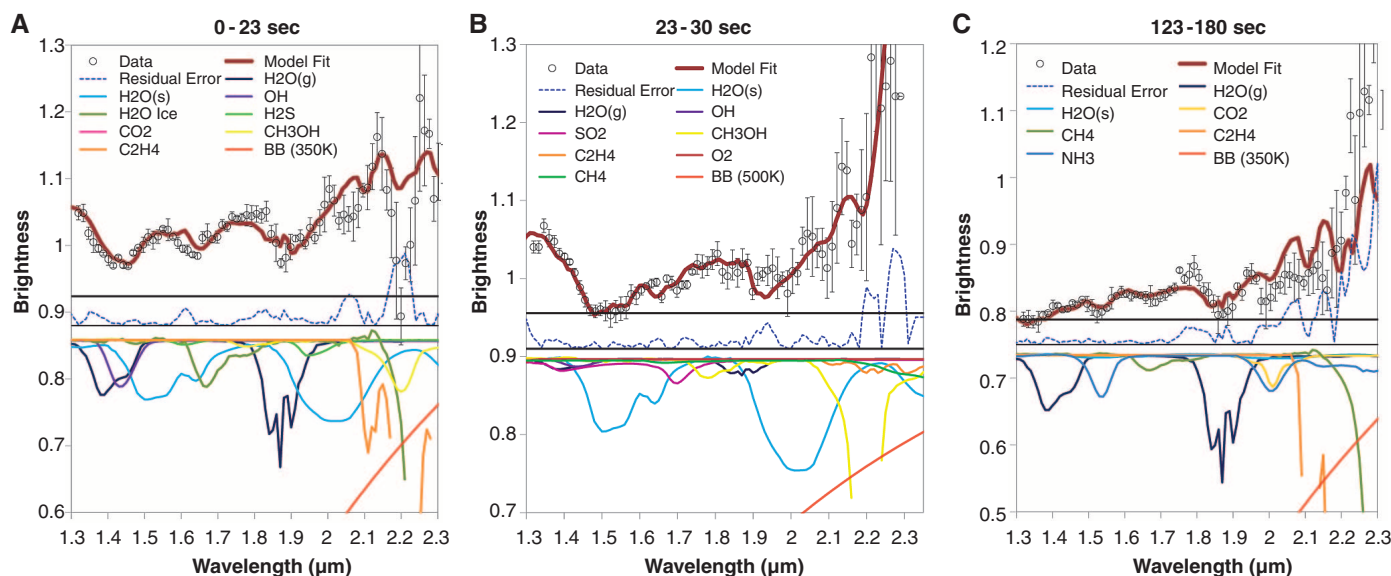


Fig. 3. (A to C) Model fits to the NIR reflectance spectra for three periods after impact. The measured spectral reflectance is shown with 1 SD error bars (measurement variance for the average of ~ 90 spectra). The fit ("Model Fit") was produced using the various volatiles indicated by the curves (each curve is normalized to water ice to show relative abundances with respect to each other) at the lower part of each figure [H₂O(g) and H₂O(s) are water vapor and water ice, respectively]. Water vapor fluo-

rescent emission is provided as a possible contribution but is not found to be necessary to fit the data within the uncertainty of the measurements. The total residual error between the observation and the model is shown (dark blue dashed line) and the 5% level indicated by a solid black line. In the wavelength range between 1.3 and 2.0 μm , residual error is typically $<1.5\%$. The χ^2/ν for each of the three fits is 1.16, 1.8, and 1.2, respectively.

narrower than mineral absorption bands, dominated the spectra. We used a linear mixing model to fit the spectra, and the quality of fit was determined by the total residual between the measurements and the fit from 1.3 to 2.0 μm —the region where water vapor and ice are primarily involved. A χ^2 minimization was performed for three epochs to elucidate the uncertainties with respect to their related confidence intervals (24). Linear fits for three of the periods shown in Fig. 2 are shown in Fig. 3. The model fit is good at wavelengths $<2.0 \mu\text{m}$, where residual errors are typically less than 1.5%. At longer wavelengths, where the instrument performance is poorest and the variance from one spectrum to the next is greatest due in part to variations in the blackbody source function, the fit is not as good. Moreover, because the NIR source function was not dominated by scattering from ejecta debris, especially over 30 to 40 s after impact, narrow gas lines were evident in the spectra and could be used to derive abundances of gaseous species. Thus, the fitting of the water features at wavelengths $<2 \mu\text{m}$ did not depend strongly on the fitting of species that absorb at wavelengths $>2 \mu\text{m}$.

In Fig. 3A, there is a strong indication of absorption by hydroxyl (OH) and water (vapor and possibly mineral bound). It is likely that OH and water gasses were part of an initial warm ($\sim 1000 \text{ K}$) vapor plume that was released just after impact and that is generally unassociated with ejecta debris—sunlight interaction. The combination of features seen at 1.4, 1.9, and 2.2 μm suggest that mineral-bound OH (at 1.4 μm), bound H_2O (at 1.9 μm), and metal-bound OH (e.g., Al-OH at 2.2 μm feature) were also present (26) but in substantially smaller quantities than gas-phase water. Figure 3B shows spectra from the period when the ejecta cloud was brightest and provides the greatest shortwave contrast for the water ice feature at 1.5 μm . This ice feature and its 2.0- μm counterpart (which is skewed to higher reflectance because of the thermal emission beginning at 1.9 μm) confirm that at least some of the water ejected from Cabeus was in the form of ice and not just water adsorbed on grains. At later times, the water vapor feature at 1.87 μm again became apparent (Fig. 3C) and persisted for the remaining duration of the 4-min observation period (compare spectra at 1.87 μm in Fig. 2).

Using the depth of the 1.87- μm absorption band in the spectrum in Fig. 3C and absorption cross sections computed using high-resolution transmission molecular absorption database (HITRAN),

the total water vapor in the instrument FOV at the time of the observation was $\sim 5.1 (\pm 1.4) \times 10^{19} \text{ molecules m}^{-2}$ (1 SD uncertainty) (24). Over the period of the observation (123 to 180 s), the FOV of the spectrometer ranged from $\sim 3.8 \times 10^7$ to $2 \times 10^7 \text{ m}^2$, giving a total water vapor mass in the instrument FOV ranging from 74 to 31 kg. Given the average FOV over this period, the water vapor mass is $53 \pm 3 \text{ kg}$ (1 SD uncertainty). Similarly, the total water ice abundance over this same period ranged from 22 to 9 kg, with an average in that time period of $16 \pm 2 \text{ kg}$ (1 SD uncertainty) (27). These are lower limits, because the ejecta cloud filled the spectrometer FOV $\sim 20 \text{ s}$ after impact, and thus some water would be outside the instrument's FOV. In a similar manner, total water vapor columns were calculated at earlier times (Fig. 3, A and B). Using the 1.5- μm ice feature shown in Fig. 3B, we estimate using a 2- μm ice particle radius (28) that the water ice grain column number is 6.3×10^7 particles m^{-2} or $\sim 131 \pm 8 \text{ kg}$ of water ice (Table 1).

The spectra evolution (Fig. 2) suggests that there was an initial release of vapors, including water vapor and OH, and small amounts of ice-rich ejecta that quickly (within $\sim 20 \text{ s}$) passed out of the instrument FOV. This initial plume was then followed by the emergence of water ice grains that continued to sublime for the entire 4-min period of observations and possibly continued sublimation from the heated surface near the impact site. This interpretation is supported by the consistent increase in the 1.86- μm water vapor absorbance after impact (Fig. 4).

The other line of evidence for water is the detection of emission associated with hydroxyl radical (OH). Hydroxyl radical may be produced through the photodissociation of water vapor or desorption of OH from grains. OH emits near 0.308 to 0.310 μm either due to excitation concomitant with photodissociation (prompt emission), or through fluorescent scattering (30) [see SOM]. The OH feature is evident in the ratio of postimpact scans to a preimpact reference composed of the average of the last four UV/Vis scans just before impact (Fig. 5). We calculated the strength of the emission line at 0.3093 μm for each postimpact ratio (from Fig. 5); the local continuum was calculated as the local average between 0.304 and 0.314 μm (Fig. 6). Before impact, there was no OH band, within measurement uncertainty. The band strength increased to an initial peak about 30 s after impact. This rise time and peak are consistent with

either photo-dissociative OH production in an expanding water vapor cloud with a temperature of about 1000 K (31) or the fluorescence of OH released at impact. The position and shape of the feature are more consistent with resonant fluorescence (30) (see SOM). This is also the same time period when OH absorption is evident in the NIR spectra (Fig. 3A). It is likely that the initial high-impact temperature ($\sim 1000 \text{ K}$) desorbed some OH from grain surfaces (3–5). However, if this expanding vapor cloud were the only source of OH, a rapid decline in OH emission would be expected at times longer than 30 s (32). Continued high levels of OH emission after 30 s and, in particular, the apparent rise after $\sim 120 \text{ s}$ suggests that additional sources of water vapor and possibly OH were present then. In particular, the position and shape of the OH emission after $\sim 190 \text{ s}$ is consistent with prompt emission (see SOM). This is also the period when NIR spectra show persistent water vapor (Figs. 2 and 3C). The LCROSS impact event resulted in a high-angle debris plume (20, 32). The persistent cloud of sunlit material allowed sustained production of water vapor (from subliming water ice) and OH (from photodissociation of water vapor).

We estimated the total OH column number, using the maximum strength of the band at impact +180 s (as this OH is most likely due to water photodissociation), as approximately $8.0 \times 10^{-3} \text{ W m}^{-2} \mu\text{m}^{-1} \text{ sr}^{-1}$, or $2.2 \times 10^{16} \text{ molecules m}^{-2}$ (33) corresponding to $23 \pm 11 \text{ kg}$ of water vapor (34). This estimate for total water vapor column from OH prompt emission strength is consistent with water vapor values derived from the NIR spectrometer for about the same time period.

The total debris mass in sunlight was estimated using UV/Vis spectrometer observations

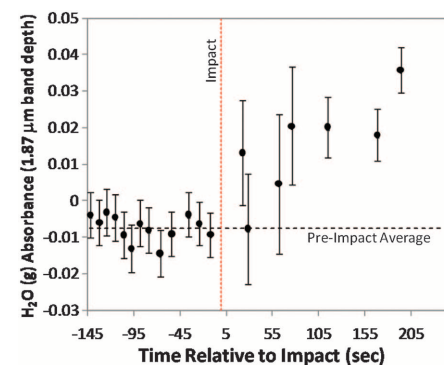


Fig. 4. The depth of the 1.87- μm water vapor band compared with the 1.77- μm shoulder in units of absorbance. Before impact, the band absorbance was calculated from averages of 30 scans. After impact, the absorbance was calculated for each of the spectra shown in Fig. 2. Also shown for reference is the approximate time of impact and the preimpact average 1.87- μm absorbance. The error bars are the root mean square of the error in radiance for each position (shoulder and band center) in the spectrum.

Table 1. Summary of the total water vapor and ice and ejecta dust in the NIR instrument FOV. Values shown are the average value across the averaging period, and errors are 1 SD.

Time (s)	Water mass (kg)		Dust mass (kg)	Total water %
	Gas	Ice		
0–23	82.4 ± 25	58.5 ± 8.2	3148 ± 787	4.5 ± 1.4
23–30	24.5 ± 8.1	131 ± 8.3	2434 ± 609	6.4 ± 1.7
123–180	52.5 ± 2.6	15.8 ± 2.2	942.5 ± 236	7.2 ± 1.9
Average	53 ± 15	68 ± 10	2175 ± 544	5.6 ± 2.9

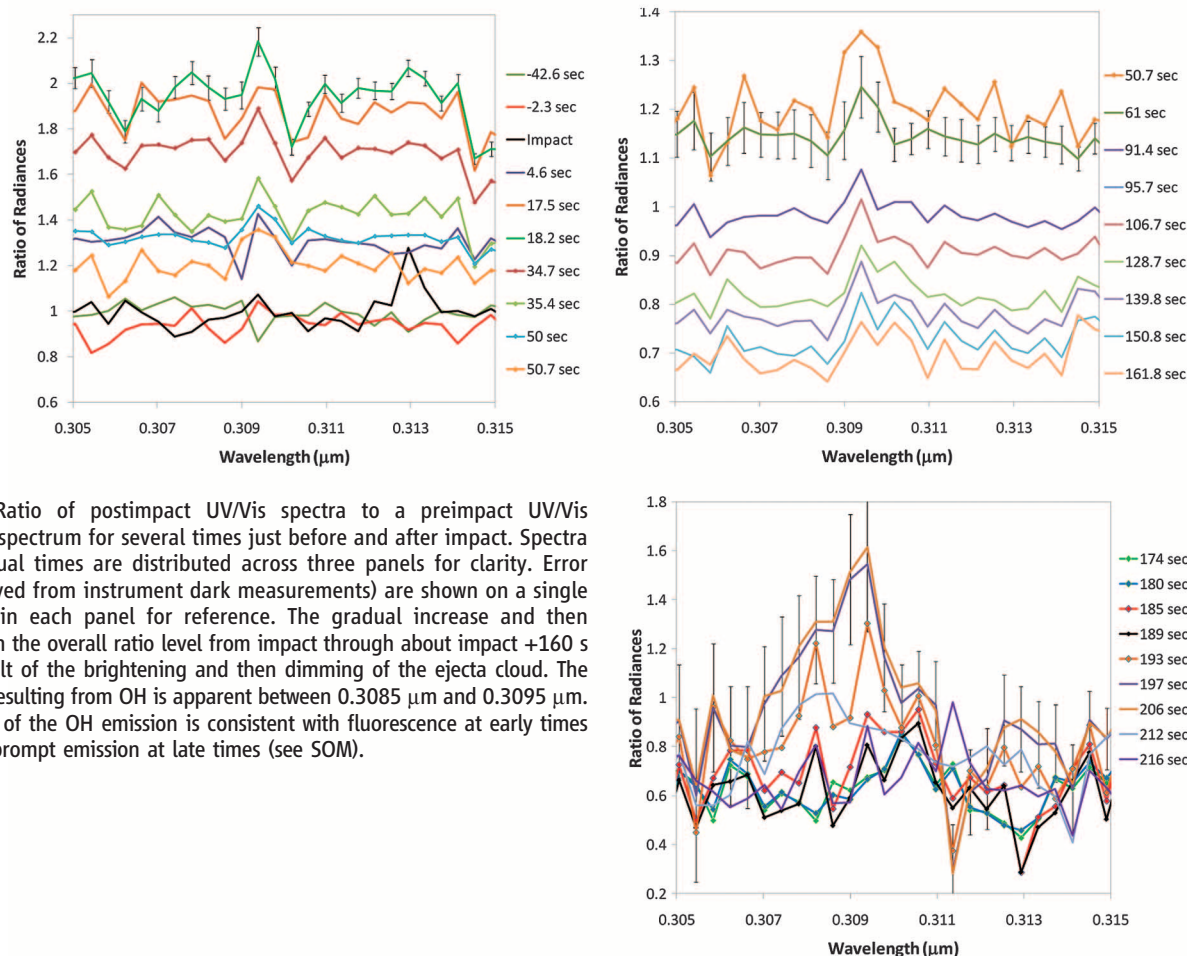


Fig. 5. Ratio of postimpact UV/Vis spectra to a preimpact UV/Vis reference spectrum for several times just before and after impact. Spectra at individual times are distributed across three panels for clarity. Error bars (derived from instrument dark measurements) are shown on a single spectrum in each panel for reference. The gradual increase and then decrease in the overall ratio level from impact through about +160 s is the result of the brightening and then dimming of the ejecta cloud. The emission resulting from OH is apparent between 0.3085 μm and 0.3095 μm . The shape of the OH emission is consistent with fluorescence at early times and with prompt emission at late times (see SOM).

of the ejecta cloud brightness as a function of wavelength. This quantity permits us to relate the total water seen in the instrument's FOVs to the concentration of water in the regolith. These data were fit using a radiative transfer model (27). Using the highest levels of radiance from the cloud measured shortly after impact (before ~30 s after impact) (Fig. 1) and a fit using an area-weighted grain radius of 2.5 μm results in a visible ($\lambda = 0.5 \mu\text{m}$) optical depth of 0.002 to 0.003. For a uniform distribution along the line of sight, these values for the optical depth imply a number density of approximately 1.4×10^6 to 2.1×10^6 grains m^{-2} . Based on visible camera images, the ejecta cloud filled about half the spectrometer FOV at 8 s and the entire FOV at 20 s. A uniform distribution of ejecta debris across the instrument FOV results in a total ejecta cloud mass in sunlight of ~4300 to 6500 kg for the range of debris column densities. If an average area filling factor of 58% (35) is applied to account for FOV under-filling before 20 s, the total mass is approximately 2500 kg and 3800 kg for the low and high estimates, respectively, with an average mass of 3150 ± 790 kg. At times later than 30 s after impact, the total cloud brightness dropped by a factor of ~3.2 (Fig. 1) and remained fairly constant for another ~200 s. During this period, the

cloud optical depth was $\sim 9.3 \times 10^{-4}$ (for a linear scaling with measured brightness), corresponding to a cloud mass of 1850 kg. This estimate of optical depth is consistent with the optical depth derived by LRO Diviner observations for the period ~90 s after impact (23). Using the estimates of the ejecta cloud mass and water from just after impact estimated for the instrument FOVs at the beginning and end of the averaging period (0 to 23 s, with the FOV filling factor applied), the total water mixing ratio in the ejecta cloud was between 4.1 and 5.1% by mass, with an average over the period of $4.5 \pm 1.4\%$ (Table 1). It is difficult to differentiate water vapor that sublimated from water ice grains in sunlight from water vapor sublimated from heated surfaces in and near the crater; thus, these estimates for the total mixing ratio of water in the ejecta cloud, based on the mass of ejecta observed in sunlight, are likely to be overestimates during this early period when the ejecta cloud was smaller than the instrument FOV. If only water ice as derived from the 23- to 30-s period (135 kg on average) and the estimates for the total ejecta cloud mass in the instrument FOV are used, the range of mixing ratios of ice in the ejecta cloud over this period was 5.2 to 5.5% by mass, with an average of $5.4 \pm 1.4\%$ by mass. Using all deri-

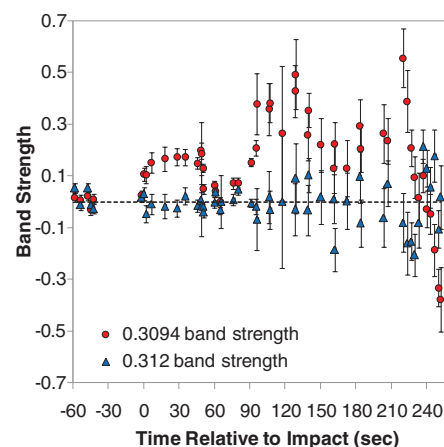


Fig. 6. Calculated band strength at 0.3094 μm (OH emission) and 0.312 μm (nearby off-band wavelength for comparison) as a function of time relative to impact. The error bars show the ± 1 SD variance across the continuum used to calculate the band depth. The 0.3094- μm band shows a clear rise in strength just after impact that remains above the preimpact baseline (dashed line) for the remaining 4-min observation duration, while the off-band measurement at 0.312 μm stays at the baseline (within error estimates).

Table 2. Abundances derived from spectral fits shown in Fig. 3. The uncertainty in each derived abundance is shown in parenthesis [e.g., for H_2O : $5.1(1.4)\text{E}19 = 5.1 \pm 1.4 \times 10^{19} \text{ cm}^{-2}$] and was derived from the residual error in the fit and the uncertainty in the radiance at the appropriate band center.

Compound	Molecules cm^{-2}	% Relative to $\text{H}_2\text{O}(\text{g})^*$
H_2O	$5.1(1.4)\text{E}19$	100.00%
H_2S	$8.5(0.9)\text{E}18$	16.75%
NH_3	$3.1(1.5)\text{E}18$	6.03%
SO_2	$1.6(0.4)\text{E}18$	3.19%
C_2H_4	$1.6(1.7)\text{E}18$	3.12%
CO_2	$1.1(1.0)\text{E}18$	2.17%
CH_3OH	$7.8(42)\text{E}17$	1.55%
CH_4	$3.3(3.0)\text{E}17$	0.65%
OH	$1.7(0.4)\text{E}16$	0.03%

*Abundance as described in text for fit in Fig. 3C.

variations of total water concentration and the errors from individual measurements for each of the three periods shown in Fig. 3, the mean water concentration is $5.6 \pm 2.9\%$ by mass (36). These high values suggest that a substantial amount of the hydrogen observed by LPNS and LEND was in the form of water ice in the crater. LEND neutron data suggests that the floor of Cabeus contains >2 weight percent water (18), or about a third the value derived from LCROSS. Several important caveats need to be considered: (i) the LEND result is strongly model dependent (e.g., assumptions about the desiccated top layer thickness); (ii) the LCROSS sample depth was possibly deeper than neutron spectroscopy can effectively sample (deeper than ~ 0.7 m); (iii) the persistent conduction of impact heat into the regolith resulted in a continued release of volatiles (23); and (iv) the neutron LEND and LPNS instrument footprints were never smaller than 10 km across; thus, spatial gradients smaller than 10 km are smoothed. The LCROSS result when combined with LEND and LPNS suggests that there is some spatial heterogeneity at scales <10 km.

As implied in Fig. 3, other hydrogen-bearing compounds are also likely to be present. Observations from the Lyman Alpha Mapping Project (LAMP) on LRO suggest that ~ 140 kg of H_2 was released at impact, corresponding to an initial regolith concentration of 1.4% (31). The total hydrogen sensed by LPNS and LRO LEND would logically include not only the water measured by LCROSS's spectrometers but also H_2 measured by LRO-LAMP and any other hydrogen-containing volatiles (e.g., CH_4) measured by LCROSS.

The abundances of volatile species other than water vapor were also derived using HITRAN [local thermal equilibrium (LTE) at room temperature, 1 atm] cross sections convolved to the instrument resolution. Given the caveat that the LCROSS impact plume contained low-density and rapidly cooling gases, probably far from equilibrium, the abundances are derived and given (Table 2). Of interest is the indication from this preliminary analyses that some volatiles other than water are considerably more abundant

(some by orders of magnitude) than the ratios found in comets, in the interstellar medium, or predicted from gas-gas reactions in the protoplanetary disk (37). Rather, the $\text{H}_2\text{S}/\text{H}_2\text{O}$, $\text{NH}_3/\text{H}_2\text{O}$, $\text{SO}_2/\text{H}_2\text{O}$, and $\text{CO}/\text{H}_2\text{O}$ (31) are more commensurate with the abundances of those molecules in molecular hot cores (38). In hot cores, gas-phase abundances are enhanced by the release of molecules that have formed on cold grain surfaces in previous stages of the prenatal cloud. In the persistently shadowed region (PSR) of Cabeus, molecule formation on cold grain surfaces could enhance abundances compared with water. One can infer that the reservoir of volatiles that LCROSS struck may partly have cometary and/or asteroidal origins but probably also has volatile molecules that may have formed in situ on cold grain surfaces in the PSR. Molecules forming on grain surfaces in cold, dense molecular clouds occur primarily through neutral-neutral reactions because the gas is very weakly ionized by cosmic rays that penetrate deep into the cloud. Conversely, the solar wind and plasma environment of the lunar exosphere is expected to have some charged species.

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28. The water ice grain radius is estimated based on the band position in the spectrum shown in Fig. 3B. Submicron ice grains will have a band center less than 1.48 μm , and for a 15- μm grain radius the band center is ~ 1.54 μm (29). The 1.5- μm band center is at ~ 1.51 μm , which is consistent with an ice grain ~ 2 μm .
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33. A g factor of 5×10^{-4} photons/s was used in the OH number estimate.
34. The uncertainty in the water vapor mass derived from the OH prompt emission at ~180 s after impact is based on the total uncertainty in the measured OH radiance at that time. The total uncertainty in OH radiance is based on the calibration error (<10%), instrument noise (from dark measurements at specific integration time), and uncertainty in the g factor (about 20%).
35. At 8 s after impact, the diameter of the ejecta cloud in the visible camera was ~4.5 km, and the FOVs of the NIR and UV/Vis spectrometers were ~10 km in diameter. At 20 s, the ejecta cloud diameter was ~8.5 km and the FOVs of the spectrometers were approximately 9.6 km in diameter.
36. The total error is the propagated error for the masses of water vapor plus water ice divided by the dust mass, using the errors (1 SD uncertainties, quoted in Table 1) in the derived masses of each component.
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39. We thank the LCROSS Project and NASA's Exploration Systems Mission Directorate (ESMD) and NASA Science

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6003/463/DC1
SOM Text
Figs. S1 to S11
References

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REPORTS

The LCROSS Cratering Experiment

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As its detached upper-stage launch vehicle collided with the surface, instruments on the trailing Lunar Crater Observation and Sensing Satellite (LCROSS) Shepherding Spacecraft monitored the impact and ejecta. The faint impact flash in visible wavelengths and thermal signature imaged in the mid-infrared together indicate a low-density surface layer. The evolving spectra reveal not only OH within sunlit ejecta but also other volatile species. As the Shepherding Spacecraft approached the surface, it imaged a 25- to 30-meter-diameter crater and evidence of a high-angle ballistic ejecta plume still in the process of returning to the surface—an evolution attributed to the nature of the impactor.

Prior studies from instruments on the Apollo (1) and Lunar Prospector (2) missions indicated the presence of mobile volatiles on or around the Moon. Multiple spacecraft recently confirmed these observations through direct spectroscopic measurements of OH and H₂O (3–5). In contrast, the Lunar Crater Observation and Sensing Satellite (LCROSS) mission used a kinetic probe (the emptied stage of the Centaur rocket) to excavate H-bearing compounds from a permanently shadowed region (PSR) near the south pole of the Moon. As the Centaur collided with the lunar surface, the trailing Shepherding Spacecraft (SSc) measured the evolution and composition of the resulting ejecta with a series of instruments. A separate contribution specifically examines H-bearing molecular species observed with LCROSS instruments (6). Here, we describe the Centaur collision with implications for the ejected mass, excavation depth, and regolith composition.

The mid-infrared cameras (MIR1 and MIR2) recorded the “first light” in the frame coinciding with the moment of impact that remained visible for the next 10 s (Fig. 1A). At that time, the resolution of the MIR instrument was approximately 1 km/pixel. Consequently, the thermal radiance generated just by the heated crater floor should have covered less than a single pixel at

that range; instead, it spanned multiple pixels corresponding to 3 to 4 km in diameter before fading with time.

The Total Luminance Photometer (TLP) was designed to detect a sudden change in brightness over visible wavelengths from a distance of more than 600 km (7), but this signal has not yet been unequivocally identified. Nevertheless, the Near-Infrared Spectrometer (NSP1), covering longer wavelengths, operated in a flash mode at 72 Hz (five wavelength channels plus a dark measurement measured every 13.8 ms); hence, it functioned as a thermal flash detector (Fig. 1B). This instrument recorded a 0.4-s rise in background intensity, followed by a 0.7-s decay. The onset for the rise in radiance in the NSP1, however, was delayed about 0.3 s from the moment of impact, on the basis of our analysis of flight data for the trajectory and topography.

The UV/VIS Spectrometer (VSP) captured both emissions from the impact flash and ejecta rising into sunlight. A large number of weak [but significant (26)] emission lines emerged within the first 0.8 s after impact, during the flash mode of the NSP1 instrument. The spectral resolution of the VSP is better than 1 nm, as demonstrated through clear identification of fine structure in the solar spectrum due to scattered light. Although many emission lines have not yet been identified with confidence, possible identifications include CN, NH, NH₂, CO₂⁺, and CS (8). The overall radiance levels increased dramatically during the next exposure (1.1 to

3.1 s after impact), indicating the arrival of ejecta into sunlight. Prominent emissions at 598 nm (Na) and a line pair at 328 and 338 nm (possibly Ag) also emerged, along with other species, such as H₂S and H₂O⁺.

The Visible Camera (VIS) started its sequence ~8 s after impact, well after ejecta had reached sunlight (Fig. 2A). The ejecta cloud increased from ~4 km (8 s) to ~8 km (20 s) in diameter and remained visible for about 42 s before dropping below the sensitivity threshold of the instrument (Fig. 2B). Both near-infrared cameras (NIR1 and NIR2) also captured the expanding ejecta cloud well after (~8 s) the moment of impact but with less dynamic range than the visible camera, thereby limiting their use for comparing dimensions.

In the final 10 s before impact, changes in exposure time and pixel gain for the NIR2 camera allowed the lunar surface to be imaged from scattered light off nearby relief. As a result, “shadowed” and “illuminated” areas are opposite to the direction of direct solar illumination (Fig. 3A). These images (higher in spatial resolution than the MIR images) reveal a region around the point of impact that is typical of the lunar surface: an undulating but relatively flat surface with few large craters. The last three frames from NIR2, starting at a range of 11 km above the surface, included a feature that correlates with a small thermally warm region in the MIR. This region is identified as the crater through correlation produced by the Centaur impact and its surrounding ejecta through correlation of telemetry and registration with the hot spot located in the MIR data. Just before the SSc collision, the NIR2 camera also recorded a diffuse disk-like feature that moved through the field of view in four successive images during approach (Fig. 3B). This feature appeared suddenly as a result of the camera exposure and gain settings.

The evolving ejecta cloud and final crater produced by the Centaur impact place constraints on the nature of the surface, the depth of any released volatiles, and context for observations by other instruments. The prolonged spectral radiance in the NSP1 data as well as NIR1 and the MIR images establish that the Centaur impact

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fully coupled with a particulate surface, not bedrock. An impact into bedrock would have resulted in a sudden rise in intensity coincident with the moment of impact, a much shorter thermal signal in the MIR, a short-lived ejecta cloud, and much less total ejected mass than that recorded by the NSP and VIS instruments.

The absence of an obvious impact flash in the TLP data is attributed to properties of the target. First, the highest-temperature materials occur at the contact between the Centaur and the regolith. In highly porous (>70% porosity) targets, material is compressed and driven downward, partly masking the view from above (9–11). Second, energy partitioned into visible light is suppressed by phase transitions in porous volatile-rich tar-

gets, even for much higher-speed impacts (10). Third, rapid quenching due to the heat capacity of the very cold 37-K regolith (12) would further suppress emitted light in visible wavelengths.

Both MIR cameras, however, detected a large heated region immediately after impact (~1 s) that persisted in successive images. At the distance of the SSc, such a large source region could not be the result of impact-heated crater floor or near-rim materials because both would be much smaller than a single pixel. This heated area is also inconsistent with the gradual thermal decay in the NSP (Fig. 1B). Consequently, the thermal source in the MIR images is attributed to impact-heated, low-angle ballistic ejecta remaining below the solar horizon over the first second (13).

During the first 0.8 s, heat generated by the Centaur impact also resulted in numerous transient emission lines in the VSP spectra. One example is the prompt emission of OH (313 nm) from the breakdown of H₂O by thermal dissociation or excited OH desorbed from grain surfaces. Because this line disappears in subsequent exposures, it is attributed to heat created as the Centaur first made contact with the surface. Because of the low impact speed, molecular species that first appear (0.8 s) must have been weakly attached to near-surface regolith grains. Impact-liberated volatiles would rapidly expand away from the nascent ejecta plume.

Numerous other emission lines emerged within the first 0.8 s and increased in strength with time

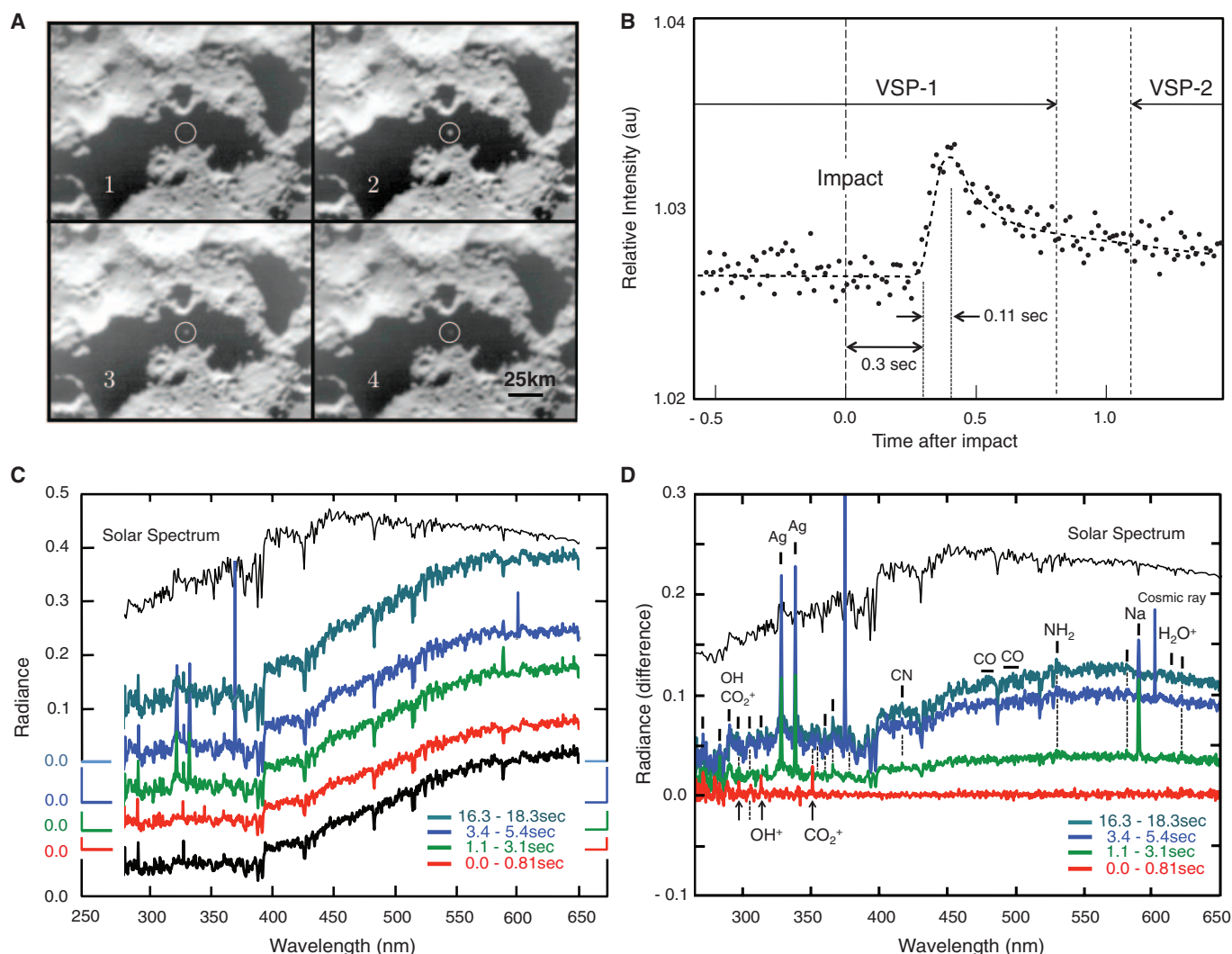


Fig. 1. (A) Evolution of the impact flash from MIR2 starting at about 1 s before impact (top left) in 2-s increments. (B) Evolution of the impact flash from the NSP-1 spectrometer in flash mode (intensity shown in arbitrary units). The moment of impact occurred 0.3 s before the rise in signal. The first exposure bracketing the impact in the VSP (VSP-1) also captured the flash. The beginning of the second exposure (VSP-2) is also shown. (C) Spectra from the Visible Spectrometer before and after the Centaur impact (all 2-s exposures): ~1.2 s before impact (black); 0.0 to 0.8 s after impact (red); 1.1 to 3.1 s (green); 3.4 to 5.4 s (blue); and 16.3 to 18.3 s (gray). The solar spectrum (taken from above the Earth's atmosphere) is shown at top. Absorption lines

(Fraunhofer lines) at this time result from sunlight reflecting off the ejecta cloud as it rose above the shadows. Spectra are offset vertically for clarity (colors on ordinate correspond to colors in figures). (D) Differences in spectra from the VSP referenced as shown in (A). The first spectrum after impact indicates several emission lines in the UV (arrows). The overall radiance difference increased in the visible wavelength range as ejecta emerged into sunlight after the end of the first exposure (green). The isolated strong line near 375 nm (and probably 601 nm) resulted from a cosmic ray hitting the charge-coupled device of the VSP detector. Dashed lines connect emissions over successive exposures, which are described in more detail in (8).

(Fig. 2). These atomic and molecular species are interpreted as coming from the regolith, rather than any residual propellant in the Centaur (14). Such a contribution should have rapidly dispersed below the detection limits. Instead, most NH and NH₂ emission lines persist or strengthen as greater

amounts of ejecta reach sunlight. This is more consistent with a contribution from a lunar regolith source.

Between 1.1 and 3.1 s after impact, the increasing wavelength-dependent radiance and deepening Fraunhofer absorption lines both indicate

sunlight scattered off ejecta above 833 m (Fig. 2). At the same time, strong emission lines attributable to Na and possibly Ag also emerged. The delayed appearance of Na is enigmatic because of its low excitation energy, even at the relatively low speed of the Centaur impact. The

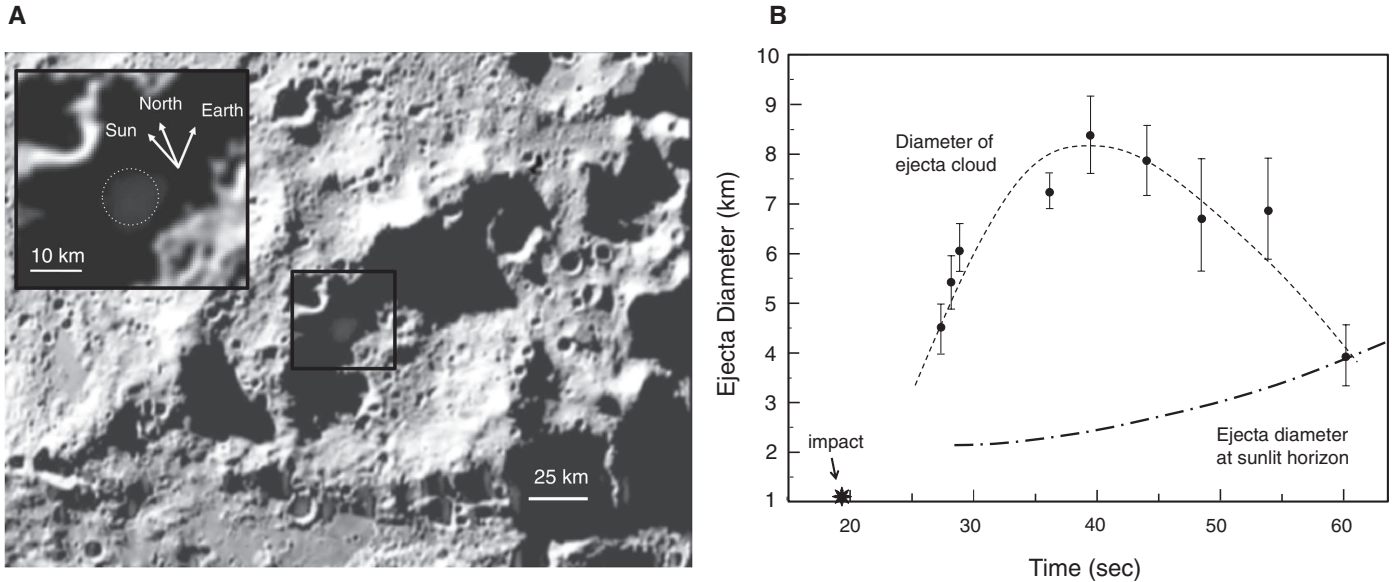


Fig. 2. (A) Image from visible camera (VIS) showing sunlit ejecta about 20 s after impact. Inset shows a close-up with the direction of the Sun and the Earth (indicated by arrows). Asymmetry of the ejecta reflects, in part, the projected shadow over the crater (from the edge of Cabeus) and across the ejecta cloud. Dotted circle represent the fields of view of the visible and near-infrared spectrometers. **(B)** Evolving diameter of ejecta from the VIS camera as a function of Coordinated Universal Time (UTC) beginning 11:31:XX (XX corresponding to seconds on the abscissa where

11:31:27.093 UTC is the time stamp of the first frame). Error bars indicate the range of values measured from a given image. VIS camera began its sequence ~7.8 s after impact noted on plot. The dash-dotted curve represents the expected diameter for the base of the ring of a nominal ejecta curtain as it falls into the shadows [as predicted by (20, 26)]. Ejecta velocity distribution is scaled to LCROSS dimensions and ballistically projected (assuming a nominal 45° ejection angle) to the sunlight horizon above the impact site.

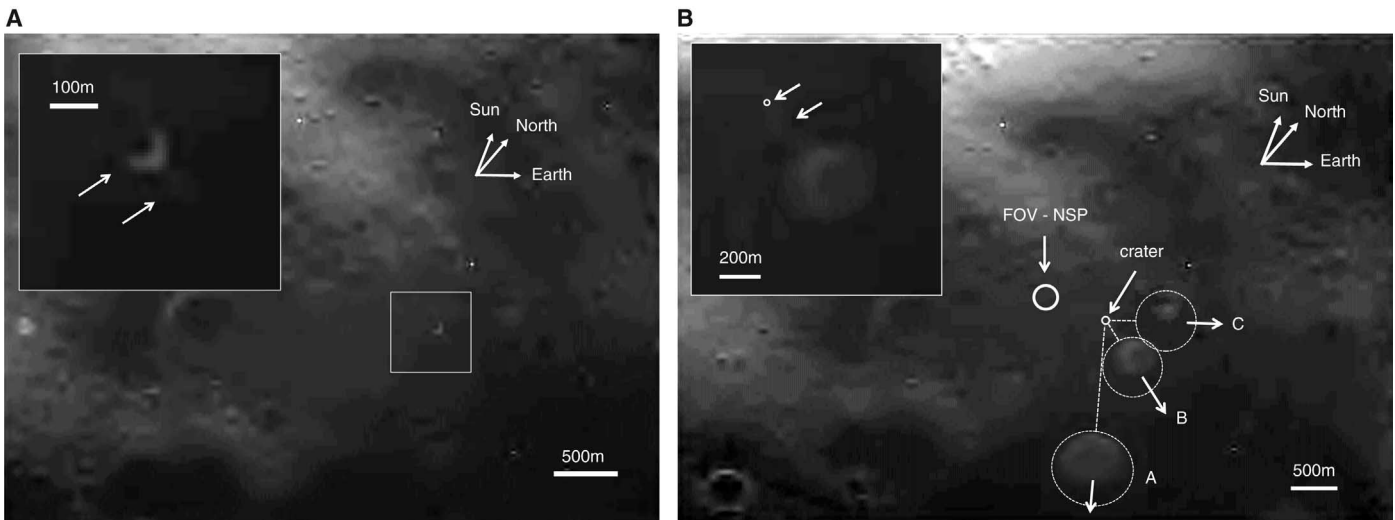


Fig. 3. (A) Centaur crater captured in the NIR2 camera 10 km above the surface. Inset shows close-up of crater (top arrow) with extension to the bottom right (bottom arrow). Because of the NIR2 wavelength range (0.9 to 1.7 μ m), brighter areas are either higher in albedo or relatively warmer. Consequently, the crater is interpreted as the darker area in the center (25 to 30 m in diameter) and is either darker or cooler than the surroundings. **(B)** Diffuse elliptical feature (inset) associated with Centaur impact crater (open circle) in the permanently shadowed floor of Cabeus crater. Scattered sunlight off the sun-facing Cabeus wall (Fig. 1A) produced sufficient light for

this NIR2 image (taken 13.75 km above the surface). The Sun-facing side of the diffuse feature, however, is brighter and indicates direct sunlight off an ejecta cloud. Dotted ellipses outline the cloud in prior (small inset below) and following frames (above, to the right). Scale for the inset (far left) is adjusted to 833 m above the surface as viewed from the SSC. Dashed lines and arrows represent surface normals extending from the Centaur impact crater (dot) to the each feature. The location of the crater is not directly below ejecta because of the offset trajectory of the trailing SSC. Filled circle indicates location and field of view of the VSP and NSP spectrometers.

Na emission could have arisen from either the target or the Centaur impactor. Na has been identified in the lunar atmosphere (15); consequently, it is an expected constituent of the regolith along with other cold-trapped volatiles. Because the Na appeared later, either the Na atoms were depleted near the surface or a layer of other volatiles partly shielded them from first-contact heating (10). The elevated Na line faded after 5 s, whereas weak Na emission filled the solar absorption line 40 s after impact. This suggests a concentration of volatile Na (and perhaps Ag) near but not on the surface.

Alternatively, the Na could have come from the Centaur. The pair of emission lines at 328 and 338 nm parallels the evolution of the Na line. Because the low impact speed would have induced little to no shock melting, most of the thermal signal could have arisen from frictional shear heating. During penetration, the Centaur

surface would have been scoured and initially mixed with the ejected regolith. In this interpretation, the Na and line-pair emissions would be tracing the evolution of near-surface materials. A source of Na or Ag in the Centaur, sufficient to produce emission lines, however, has not been identified.

Most emission lines developed between 1.1 and 3.1 s and strengthened with time (Fig. 2). Several other lines appeared during the next exposure (3.4 and 5.4 s): 405.3, 426.8, and 461.4 nm (8). Although most lines strengthened with time, the strong emission line at 405.3 nm appeared only once. Another emission (OH at 290.4 nm) appeared 3.4 s after impact, became stronger, and then faded by 18.3 s. A line coincident with H_2O^+ (313 nm) also became prominent after 16.3 s. This evolving pattern illustrates changes in concentrations with depth or a time delay between heating and sublimation once exposed to sunlight (6).

The diffuse elliptical feature moving across four successive NIR2 images near the end of transmission (Fig. 3A) is interpreted as high-angle ($>80^\circ$ launch angle from the horizontal) ballistic ejecta still returning to the surface. The SSc struck the lunar surface 3 km southeast of the Centaur crater. As the SSc approached the surface, perspective would have caused a sunlit ejecta cloud 833 m above the crater to move across the background. Modeling of a suspended disk at the sunlight horizon in the field of view of the NIR2 during the final approach is generally consistent with the motion of this feature.

The apparent evolution of the shape of the feature and its curved path is attributed to the changing perspectives of the sunlit portion of the cloud during SSc approach (Fig. 3A). The changing dynamic range of the imaged field of view controlled when this cloud could first be detected (Fig. 3B, bottom inset). At this time, the SSc was still well above the surface and viewed the ejecta cloud nearly in line with the crater. The sunlit ejecta then formed a nearly circular cloud, ~ 820 m in diameter. When the crater appeared in the next frame, the impact point, ejecta cloud, and SSc were no longer directly in line. As the SSc approached, the cloud in the next image became fainter, smaller, and more offset. The solar phase angle (the angle between the Sun, ejecta cloud above the surface, and spacecraft) increased with decreasing altitude, and the SSc viewed shadowed ejecta. As a result, the center of the brightest portion of the cloud faded and shifted in the last moments as the SSc moved over the crater.

The dark central area in the last three transmitted NIR2 images is interpreted as the actual crater (about 25 to 30 m across). The darkness may represent an exposed colder substrate (perhaps enhanced by the very low heat capacity of aluminum debris from the Centaur) or represents a lower albedo. The slightly brighter crescent-shaped area indicates either a higher albedo near-rim ejecta or slightly warmed ejecta

due to latent heat released during phase changes, such as condensation on the ejecta.

The size of the Centaur impact crater is consistent with predictions, but the evolution of the ejecta requires explanation. Before the encounter, the total mass of ejecta predicted to reach an altitude of 2 km (or higher) ranged from 6×10^3 to $>10^6$ kg (16). Moreover, a canonical ejecta curtain would form an expanding annulus as the ejecta returned into shadow (17). Even though the inner ring of the annulus should have been resolvable (Fig. 2B, dash and dotted line), the ejecta annulus never emerged; rather, ballistic ejecta formed a diffuse and relatively symmetric cloud throughout.

Recent laboratory experiments revised limits on the amount of the ejecta reaching sunlight from the Centaur impact. Solid projectiles striking a lunar regolith-like particulate surface at 2.5 km/s (Fig. 4A) initially produce ejection angles of $\sim 30^\circ$ (18) before evolving into nominal ejection angles of $\sim 45^\circ$ (19, 20). The emptied Centaur rocket, however, was effectively hollow, with a total density of about 0.03 g/cm³. Impact experiments by means of hollow projectiles into a regolith-like target result in a very different ejection sequence (Fig. 4B). In addition to an early-time low-angle ejecta component (18), a high-angle ejecta component emerged initially at high speeds (>1 km/s), decreasing with time (21). Such a high-angle ejecta component would account for the absence of the TLP signal, the diffuse cloud in the VIS images (Fig. 2), the inferred ejecta cloud in the NIR2 (Fig. 3B), the long-lasting spectral signatures (VSP and NSP), and the elevated radiance throughout nearly the entire approach (6). Telescopic observations of such a cloud would have been challenging because of the low optical depth in that orientation (22).

Ejecta still in sunlight for more than 4 min (composing the cloud about 820 m in diameter) represent material ejected at nearly vertical angles at speeds greater than 400 m/s. This component would not follow the standard mass-velocity scaling relations (20, 23). In laboratory experiments, hollow spheres failed to eject tracers placed one projectile diameter below the surface. Consequently, the observed concentrations of volatiles (6) must be considered highly conservative values and would depend on their distribution with depth in the regolith.

In summary, our results show that a volatile-rich and porous regolith resulted in a fainter-than-expected impact flash in the visible spectra but a longer-than-expected flash in the near-infrared. Low-angle incandescent ejecta resulted in a larger-than-expected feature in the mid-infrared. Although the observed ejecta plume in the visible spectra never developed into the expected sunlit ejecta ring, ejecta did remain evident in both the VSP and NSP. This was long after the canonical outward-moving ejecta curtain should have disappeared below the solar horizon. High-angle ballistic ejecta were still returning as the SSc

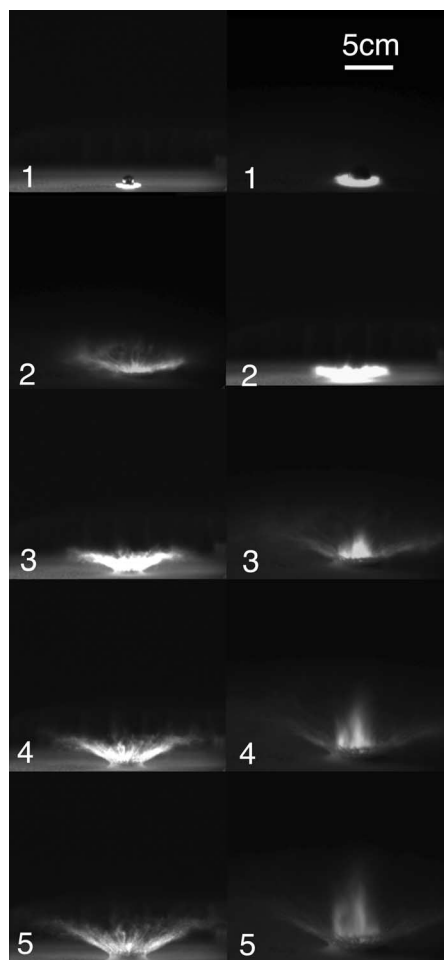


Fig. 4. Contrast in evolution of high-velocity ejecta caused by experimental impacts by (left) solid and (right) hollow aluminum projectiles at about the same speed as the LCROSS impact (2.5 km/s). Just after the moment of impact (frame 1, right), the hollow projectile (1.27 cm in diameter) temporarily obscured the heated contact surface. This impact generated a near-vertical ejecta plume, in contrast with the solid sphere.

approached the surface and contributed to sunlit volatile species detected in the NSP throughout approach. LCROSS provided a close view of excavated ejecta and volatiles, whereas the Lyman Alpha Mapping Project (LAMP) had a different viewpoint detecting other species (CO, Ca, Mg, and Hg) rising above 7 km (24). The Centaur impact produced a crater about 25 to 30 m across, with about 4000 to 6000 kg of ejecta reaching sunlight. Going by the evolving spectra, the impact released a variety of volatiles besides water, which suggests a variety of possible sources besides solar wind implantation (25).

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7. The TLP covered a spectral range from 350 to 975 nm with a wide field of view (11°) that would have included sunlit regions. The TLP was designed, however, to detect sudden changes in light (from a 1000 K source) down to a level of about 1 nW at the detector with a signal-to-noise ratio (SNR) > 5 . The intensity and evolution of the flash in this unit provides constraints about the nature of the struck surface. Preimpact laboratory impact experiments over a range of impact speeds into particulate targets yielded predictions for luminous efficiencies of 10^{-5} to 10^{-6} (ratio of luminous flux to impact energy) for the LCROSS impact speed with initial temperatures of <2500 K (9).
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14. As part of the mission design, the Centaur upper stage had been purged for more than 3 months during its orbit around the Earth (6). Mission engineers have estimated that less than 3 kg of propellant [from the O_2 , H_2 , and hydrazine (N_2H_4) tanks] remained with other volatile components in the Centaur (insulating foam and paint), contributing an additional 35 kg (6). The impact speed would not fully vaporize this material, except scoured surfaces directly in contact with the regolith during penetration. Even then, only a small fraction of this component would have been ejected at (or excited to) sufficient speeds to reach sunlight.
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16. Estimates for the ejected mass covered a wide range depending on models and assumptions. Extrapolations from late-stage scaling relations (for 45° ejection angles) predicted that only about one projectile mass (~ 2000 kg) or about 1% of the total ejected mass) would reach an altitude of 2 km (21, 26), which is about an order of magnitude less than estimates from smooth-particle-hydrocode models (20). For standard ejection models (23), one projectile mass is probably an overestimate because such extrapolations do not include the effect of early time-energy losses (compressible target) and the nature of the impactor (hollow). A high-angle plume, however, substantially offsets this reduction. Because energy and momentum partitioning still limits the maximum ejected mass, not much more than two to three projectile masses (4000 to 6000 kg) should have reached sunlight at ~ 0.8 km. Derivations of the total ejecta mass in sunlight from LCROSS VSP and Lunar Reconnaissance Orbiter (LRO) LAMP observations are consistent with this prediction (6, 26). It also should be noted that scattering in the near infrared was five times less than in the visible, which is about a factor of two times less than expected or modeled (6).
17. The expanding annulus of ejecta arises from the evolution of ballistic ejecta. With time, ejection velocities systematically decrease, launch positions increase in distance from the point of impact, and ejection angles remain nearly constant. The evolution of ballistic ejecta in a given direction produce an inclined thin sheet called the ejecta curtain that advances across the surface away from the crater rim. In all directions, this curtain resembles an inverted cone that expands with time around the impact point. It represents the locus of individual ejecta ballistic particles at a given time, as revealed in laboratory experiments (19) and models (20).
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22. The VSP radiance flattened as its narrow field of view subsampled the sunlit ejecta cloud (6). After about 180 s, the Centaur crater (and ejecta in the foreground) was no longer directly centered, and the VSP and NSP instruments no longer had the remaining high-angle component in their fields of view (Fig. 3B). Consequently, radiance levels dropped rapidly during final approach. Nevertheless, still-returning ejecta still in sunlight continued to liberate adsorbed volatiles, which detached as an expanding, tenuous vapor cloud. Ejecta and volatiles within this narrow high-angle plume would have been difficult to detect from the Earth. Even though some ejecta did reach sufficient altitudes, the low column density and limited extent (as viewed from the side) made telescopic observations difficult.
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25. Although trapped CO, OH, and H_2O in the regolith could come from solar wind processes (3–5), other sources include recent gas release from the lunar interior (1, 15, 27) and cometary or asteroid impacts. Although the high impact speeds of cometary impacts generate gas expansion speeds exceeding the lunar escape velocity, recent models predict that a small fraction could be retained (28). Such contributions to polar cold traps from infrequent cometary sources require a replenishment rate that exceeds the loss rate caused by the continuous “gardening” by small impacts. It is also possible, however, that a cometary impact (or impacts) recently resupplied the current inventory, which will gradually disappear until the next volatile-rich collision. Two other processes can also temporarily sequester volatiles within the regolith. First, not all volatiles released (or generated) by small cometary impacts escape as expanding vapor; rather, a fraction will be injected into the regolith below the surface before migrating upward. Second, impact-trapped pockets of hydrous phases (concentrations as high as 20 weight percent) occur in both natural and experimental impact glasses (29). Such high concentrations are possible because of the high solubility of glass at high pressures and temperatures, combined with rapid quenching. In both cases, slow diffusion and subsequent impact-gardening would gradually release the entrained volatiles and allow their migration to cold traps.
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30. We thank the LCROSS Project and NASA’s Exploration Systems Mission Directorate (ESMD) and NASA Science Mission Directorate (SMD; Planetary Geology and Geophysics) for support. We are also very grateful to the NASA Ames Vertical Gun Range technical team and the Thermophysics Facilities Branch at NASA Ames for their continued support in experiments. B.H. was supported through a NASA Rhode Island Space Grant fellowship for part of this study.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6003/468/DC1
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Figs. S1 to S3
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LRO-LAMP Observations of the LCROSS Impact Plume

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On 9 October 2009, the Lunar Crater Observation and Sensing Satellite (LCROSS) sent a kinetic impactor to strike Cabeus crater, on a mission to search for water ice and other volatiles expected to be trapped in lunar polar soils. The Lyman Alpha Mapping Project (LAMP) ultraviolet spectrograph onboard the Lunar Reconnaissance Orbiter (LRO) observed the plume generated by the LCROSS impact as far-ultraviolet emissions from the fluorescence of sunlight by molecular hydrogen and carbon monoxide, plus resonantly scattered sunlight from atomic mercury, with contributions from calcium and magnesium. The observed light curve is well simulated by the expansion of a vapor cloud at a temperature of ~ 1000 kelvin, containing ~ 570 kilograms (kg) of carbon monoxide, ~ 140 kg of molecular hydrogen, ~ 160 kg of calcium, ~ 120 kg of mercury, and ~ 40 kg of magnesium.

Permanently shaded regions (PSRs) on the lunar surface have long been recognized as potential cold traps for volatiles (1–3). Although the focus has been primarily on water (because of its importance for future human ex-

ploration), the PSRs are generally cold enough to trap many other volatile species. With not even occasional direct sunlight for warmth, temperatures in PSRs are found to be 35 to 100 K, which is much lower than the ~ 200 K in the surrounding

polar regions (4). Few species (such as CO, N₂, Ar, and Ne) can sublime at such low temperatures. Furthermore, overturning of the regolith by micrometeorites (impact gardening) buries accumulated volatiles and protects them from loss due to exposure at the surface (5). Thus, PSRs have the potential to collect and store volatiles from the rest of the Moon.

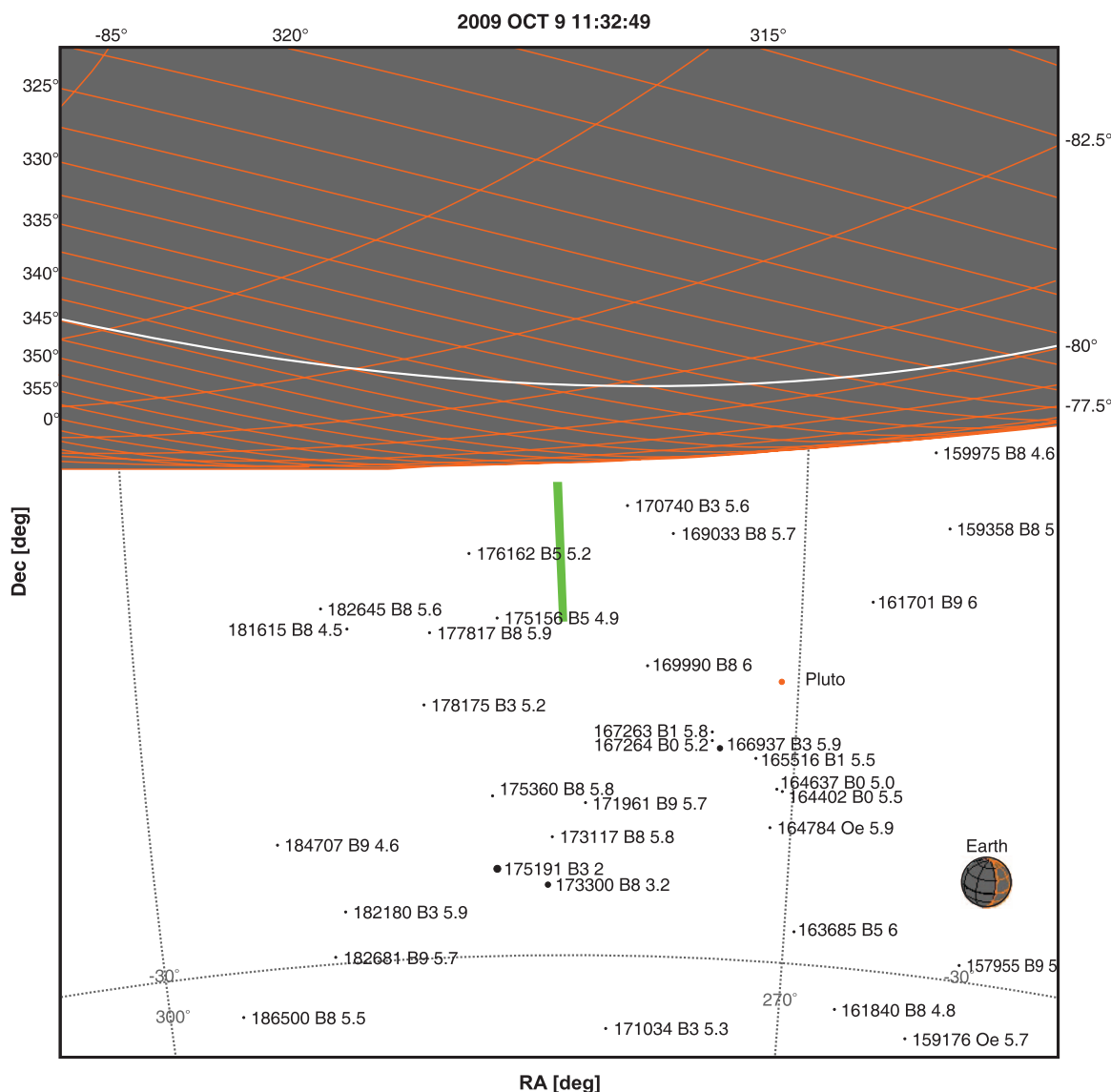
On 9 October 2009 at 11:31:19.452 UT, the Lunar Crater Observation and Sensing Satellite (LCROSS) used the Lunar Reconnaissance Orbiter's (LRO's) spent Centaur upper stage (6) as

a kinetic impactor, striking Cabeus crater on the Moon (latitude -84.675° , east longitude 311.281°). The LRO satellite (7), in a ~ 50 -km altitude polar orbit around the Moon, flew past the impact site with a closest approach at ~ 90 s after the impact, while moving toward the south pole along a nearly noon meridian ($\sim 11:34$ local time) at ~ 1.66 km/s (the LRO orbital period is ~ 113 min). The Lyman Alpha Mapping Project (LAMP) far-ultraviolet (FUV) imaging spectrograph on the LRO (8) was pointed above the limb of the Moon to look for FUV emissions associated with the LCROSS impact plume (Fig. 1). At the time of closest approach of the LRO to the impact site, the 6° -long LAMP slit was aligned vertically and centered at an angle of 80.5° from the nadir, in the plane perpendicular to the LRO velocity vector (as it was for several minutes before and after). In this location, the LAMP slit covered altitudes of ~ 7 to 38 km above the limb (~ 294 km away), corresponding to an altitude range of ~ 27 to 38 km above the impact site (~ 103 km away).

In order to better understand the sky background emissions and to track the evolution of the plume, the LAMP targeted the limb over the southern polar region for a total of eight consecutive LRO orbits: two before, one during, and five after the LCROSS impact.

After subtracting the sky background (9), using the observations from the previous orbit, the LAMP detected FUV emissions associated with the LCROSS impact (Fig. 2). These emissions arose from the resonant and fluorescent scattering of sunlight by various species lofted into the LAMP field of view. The light curve of the emissions peaked at ~ 45 s after the LCROSS impact (~ 45 s before the LRO spacecraft reached its closest approach to the impact site). At this time, the line of sight of the LAMP boresight was ~ 85 km equatorward of the impact site, implying a bulk velocity of ~ 1.9 km/s for the plume species contributing to the LAMP signal. In fact, the leading edge of the light curve began at ~ 25 s after impact, when the LAMP boresight was ~ 115 km

Fig. 1. The view from the LRO at 90 s after the LCROSS Centaur impact is shown near the south pole of the Moon, with the outline of the LAMP slit projected onto the sky shown in green. Moderately bright UV stars [of spectral types O and B, with magnitudes (V) < 6] are indicated, along with their identification numbers in the Henry Draper (HD) catalog. Pluto (orange dot) and Earth (at lower right) are also shown. On the sky, right ascension and declinations are shown by dotted lines every 30° . On the lunar surface, lines of latitude and longitude are shown in orange. The white arc shows a circle of 100-km radius on the surface, centered on the impact location (latitude -84.675° , east longitude -48.719°).



equatorward of the impact site, so that the fastest parts of the plume had a velocity of ~ 4.6 km/s. This plume velocity is surprisingly large, given that the LCROSS impact velocity was ~ 2.5 km/s (lunar escape velocity is 2.4 km/s). The 180- to 190-nm light curve had a much shorter rise and fall than the 130- to 170-nm light curve and showed a secondary peak at the time of closest approach. No signals related to LCROSS were detected by the LAMP in the five orbits after the impact orbit.

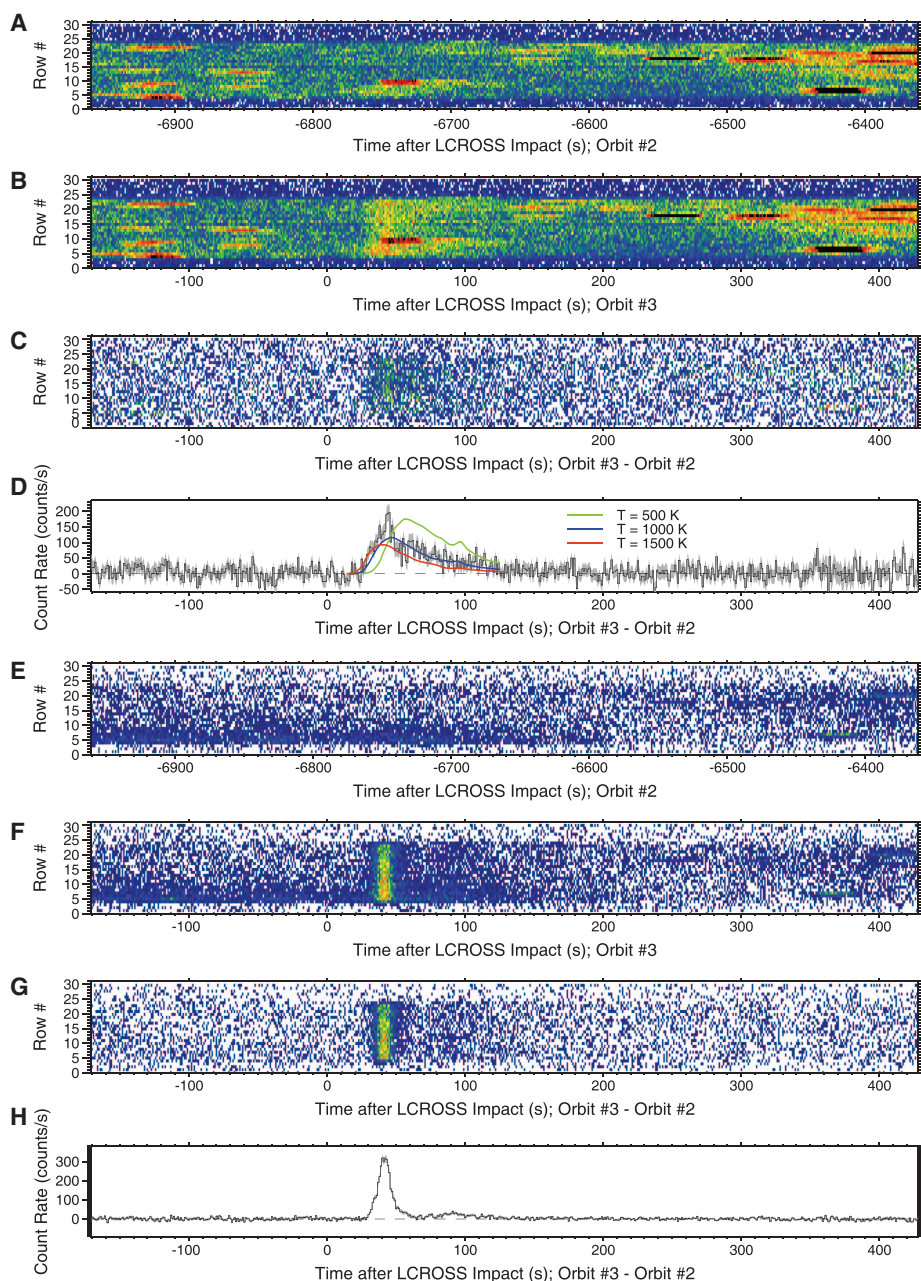
Using the results of Fig. 2, a net spectrum of the plume was extracted by integrating over the times from 30 to 60 s after the impact, when the plume was brightest in the LAMP data (Fig. 3). The net spectrum shows two main features: (i) a strong emission at 185 nm, and (ii) a broad continuum from 130 to 170 nm. The region near 121.6 nm

(Ly α) is noisy because of the large sky background, and the LAMP sensitivity is low at wavelengths <115 nm (8). The continuum emission is well fit by H₂ and CO fluorescence of sunlight (10). A model that represents most of the signal from 130 to 170 nm as fluorescence from a cloud of H₂ expanding from an initial temperature of 1000 K provides an acceptable fit to the observed continuum in the net plume spectrum (Fig. 3) and an excellent fit to the observed light curve (Fig. 2). The Monte Carlo transport model we used (11) follows a gas under the influence of lunar gravity as a function of time from the point when it becomes collisionless until it is lost from the atmosphere. H₂ molecules were injected into the exosphere from the LCROSS impact site with a velocity magnitude taken from a thermal distri-

bution and random direction. Losses due to Jeans escape and photodissociation were included, although the time scale for photodissociation is long (~ 77 days) as compared to the duration of the event. The 1000 K light curve shown in Fig. 2 is equivalent to the H₂ gas becoming collisionless at that temperature. The H₂ fluorescence model fit to the plume spectrum in Fig. 3 likewise assumes a kinetic and rotational temperature of 1000 K. The CO fluorescence spectrum also assumes a rotational temperature of 1000 K, although the dependence of the spectrum on rotational temperature is small (10). Initial attempts to fit the 180- to 190-nm light curve with an expanding cloud of Hg atoms have been unsuccessful.

In order to fit the rest of the spectrum, we estimated the contribution that could be provided

Fig. 2. Light curves for two interesting spectral regions, with images showing the count rate as a function of time (relative to the LCROSS impact) for each spatial row along the LAMP slit, and with plots showing the slit-integrated count rate. (A to D) show results for the spectral region from 130 to 170 nm, whereas (E to H) show results for the spectral region from 180 to 190 nm. (A) and (E) Light curve images from the LRO orbit previous to the LCROSS impact orbit (for which the observing geometry was identical). (B) and (F) Light curve images from the impact orbit. In each of these panels, the many ~ 40 s-long horizontal features (in yellow, red, and black) are UV-bright stars moving through the LAMP slit field of view; the bright star in rows 7 and 8 at 350 to 390 s after impact (6440 to 6400 s before impact in the previous orbit) is HD170740, a B3 star with $V = 5.6$, as noted in Fig. 1. (C) and (G) The net light curve image when the pre-impact light curve image of (A) and (E) is subtracted from the impact light curve image of (B) and (F). (C) and (G) show how well the stars and other sky background features may be subtracted out, with the remainder showing a spatially resolved light curve of the LCROSS impact plume, as the LAMP slit moves across it while it is evolving. (D) and (H) The integrated net count rate (with 1σ errors in gray) for each wavelength region over the active area (rows 5 to 23) of the LAMP slit. In (D), model results for the expected count rate (due to fluoresced sunlight) from an expanding vapor cloud consisting of 140 kg of molecular H at three different initial temperatures (T) are overplotted.



by all elements having neutral-state resonance line transitions in the LAMP bandpass (12) (the low LCROSS impact speed makes the formation of ionized species unlikely). Although many useful upper limits and marginal detections were derived (see Table 1, which shows values for the one or two brightest lines of each element having resonance lines in the LAMP bandpass, although all contributing lines were considered), the net spectrum is best fit by a combination of H_2 , CO, Hg, Ca, and Mg, all of which are plausibly expected to be plentiful in permanently shaded regions of the Moon. The large column ($1.7 \times 10^{13} \text{ cm}^{-2}$) of CO indicated by the model fit is presumably derived from solar wind C and O ions that have migrated to the permanently shaded regions. Apollo soils liberate relatively large (~ 0.01 weight percent) fractions of CO during pyrolysis at temperatures of 900 to 1200 K (13). The derived line-of-sight column density for Hg is slightly optically thick at line center ($\tau_0 \sim 0.8$ for an assumed dispersion velocity of 1.9 km/s), so it is possibly an underestimate. Assuming that the path length through the plume was the same for all species as for H_2 , the derived column densities imply that the mass of ground-state atomic Hg in the plume was $\sim 120 \text{ kg}$.

If the mass of soil that was heated to $\sim 1000 \text{ K}$ was $\sim 10,000 \text{ kg}$, as estimated (14, 15), then the mass mixing ratios of the volatiles CO, hydrogen, and Hg in the uppermost permanently shaded soil of Cabeus crater are 5.7, 1.4, and 1.2%, respectively.

The 130- to 170-nm light curve is completely consistent with the immediate formation of H_2 , and any substantial contribution of H_2 from the photolysis of water is ruled out by the strict upper limits on other photolysis products (such as H and O) obtained by the LAMP. For example, for any H_2 resulting from photodissociation, ground state O is produced in roughly equal amounts. The 2σ upper limit O column density of $8.3 \times 10^{10} \text{ cm}^{-2}$ from the 130.4-nm resonance triplet shows that photodissociation of water vapor did not substantially contribute to the column of $5.8 \times 10^{13} \text{ cm}^{-2}$ H_2 we observed. Similar conclusions follow from the 2σ upper limit H column of $4.3 \times 10^9 \text{ cm}^{-2}$. The branching ratio of water photolysis in sunlight has 88% going into $\text{OH} + \text{H}$, compared to 5% going into $\text{H}_2 + \text{O}(^1\text{D})$ and 6% going into $2\text{H} + \text{O}$ (16). So the H_2 column expected from photodissociation would be ~ 20 times less than the H column, or $< 3 \times 10^8 \text{ cm}^{-2}$, compared with the observed column of $5.8 \times$

10^{13} cm^{-2} . Thus, our hydrogen detection seems to represent adsorbed H in the local regolith.

Although the LCROSS impact was not easily seen from Earth, the LAMP, in lunar orbit on the LRO, observed a distinct plume generated by the Centaur impact near the south pole of the Moon. One of the science goals of the LRO mission is to identify and map the distribution of water ice in lunar PSRs (7) by observing reflectance characteristics of the surface with the Lunar Orbiter Laser Altimeter and the LAMP. Neither of these experiments can detect subsurface ice. Observations of the LCROSS impact plume have extended the reach of the LRO several meters into the soil and buried volatiles of Cabeus crater. Whatever form they take in the lunar soil, the carbon monoxide and molecular hydrogen detected must come out directly as CO and H_2 in order to produce the observed light curve. An initial temperature of 1000 K is enough to result in such desorption (17). Likewise, Ca and Mg are not unexpected anywhere on the Moon and are seen in Mercury's atmosphere (18). Finally, the detection of the element mercury may seem surprising, but it has long been predicted in PSRs at mass mixing ratios similar to or larger than we

Fig. 3. (A) The net LAMP plume spectrum (gray histogram) on a logarithmic brightness scale (in units of rayleighs per nanometer), using only data received between 30 and 60 s after impact and with the sky background removed by subtracting out the corresponding data (~ 113 min earlier) from the previous LRO orbit. The black histogram shows the net spectrum smoothed by a running mean of nine 0.17-nm-wavelength bins (i.e., by $\sim 1.6 \text{ nm}$), whereas the black dashed line shows the smoothed-spectrum 2σ errors. Also shown for comparison are model spectra for H_2 , CO, and all the neutral atomic species with resonance transitions in the LAMP bandpass. The spectrum for each species is labeled above the wavelength of its maximum possible contribution to the observed signal plus a 2σ error (corresponding column densities are given in Table 1). **(B)** The same smoothed LAMP spectrum (black histogram) on a linear brightness scale, overlain on $\pm 2\sigma$ errors (in gray), with a model least-squares fit for comparison (red dashed line) and with residuals shown below (in green). The reduced χ^2 statistic for the least-squares fit is $\chi^2/\nu = 0.9$ (where ν is the number of degrees of freedom). A change in scale by a factor of 6 occurs at a wavelength of 170 nm. The spectrum at wavelengths $> 193 \text{ nm}$ is suspect because of falling instrument sensitivity and detector edge effects. The contributions to the least-squares fit spectrum from H_2 , CO, Hg, Ca, and Mg are also shown, although the least-squares fit also includes all the species shown in (A). The corresponding line-of-sight column densities for each element are provided in Table 1.

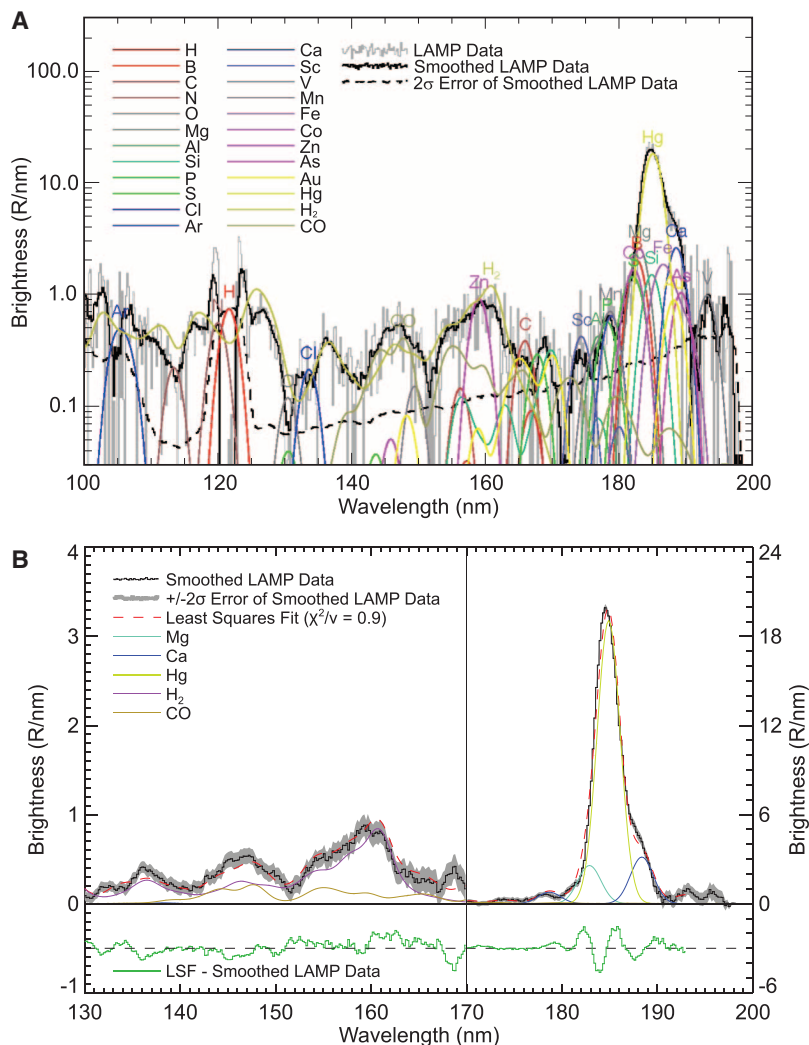


Table 1. Elemental contributions to the plume spectrum observed by the LAMP. Relevant information on each element’s brightest one or two resonance lines in the LAMP bandpass is given, including wavelength, oscillator strength, and *g*-factor. A least-squares fit or 2σ upper limits to the line-of-sight column density are given, along with the corresponding soil mass abundance (assuming a heated soil mass of 10,000 kg). These abundances are for the atomic form of each element only (except for H₂ and CO); other forms of each element may also be present in the plume. LTV, low-temperature volatile (<600 K); HTV, high-temperature volatile (600 to 1300 K); CON, early condensate; SIL, silicate; MET, metal (25). N/A, not applicable.

Element	Type	Wavelength (nm)	Oscillator strength	<i>g</i> -factor (photons s ⁻¹)	Least-squares fit column density (cm ⁻²)	Soil mass abundance* (%)
H	LTV	102.57	0.07906	3.84 × 10 ⁻⁰⁶	<4.3 × 10 ⁹	(See H ₂)
		121.57	0.41588	2.88 × 10 ⁻⁰³		
B	LTV	182.59	0.14893	9.14 × 10 ⁻⁰⁵	<3.2 × 10 ¹¹	<0.04
		182.64	0.13550	8.15 × 10 ⁻⁰⁵		
C	LTV	156.03	0.07603	3.67 × 10 ⁻⁰⁶	<6.2 × 10 ¹¹	<0.09
		165.69	0.13397	1.97 × 10 ⁻⁰⁵		
N	LTV	119.96	0.12940	9.30 × 10 ⁻⁰⁷	<3.9 × 10 ¹³	<6.6
		120.02	0.08569	5.55 × 10 ⁻⁰⁷		
O	LTV, SIL	130.49	0.04818	8.01 × 10 ⁻⁰⁶	<8.3 × 10 ¹⁰	<0.02
		130.60	0.04786	8.08 × 10 ⁻⁰⁶		
Mg	SIL	182.79	0.05495	3.20 × 10 ⁻⁰⁵	1.3 × 10 ¹² ± 5.3 × 10 ⁹	0.4
Al	CON	176.64	0.72101	2.03 × 10 ⁻⁰⁴	<2.8 × 10 ¹⁰	<0.009
		176.91	0.15415	4.91 × 10 ⁻⁰⁵		
Si	SIL	169.62	0.25170	3.32 × 10 ⁻⁰⁵	<6.0 × 10 ¹⁰	<0.02
		184.55	0.27353	1.60 × 10 ⁻⁰⁴		
P	MET	177.50	0.15415	5.05 × 10 ⁻⁰⁵	<9.3 × 10 ¹⁰	<0.04
		178.28	0.10208	3.85 × 10 ⁻⁰⁵		
S	HTV	180.73	0.12051	6.61 × 10 ⁻⁰⁵	<1.5 × 10 ¹¹	<0.06
		182.62	0.10965	6.63 × 10 ⁻⁰⁵		
Cl	LTV	116.72	0.20321	4.06 × 10 ⁻⁰⁷	<4.7 × 10 ¹¹	<0.2
		133.57	0.02500	7.38 × 10 ⁻⁰⁶		
Ca	CON	178.63	0.01016	3.99 × 10 ⁻⁰⁶	3.3 × 10 ¹² ± 1.3 × 10 ¹⁰	1.6
		188.32	0.01698	1.62 × 10 ⁻⁰⁵		
Sc	CON	173.90	0.10494	1.93 × 10 ⁻⁰⁵	<1.7 × 10 ¹¹	<0.09
		174.47	0.10136	2.24 × 10 ⁻⁰⁵		
V	CON	192.94	0.00310	4.11 × 10 ⁻⁰⁶	<4.0 × 10 ¹²	<2.4
Mn	HTV	178.53	0.01236	4.83 × 10 ⁻⁰⁶	<1.9 × 10 ¹²	<1.3
		178.55	0.00912	3.57 × 10 ⁻⁰⁶		
Fe	MET	185.17	0.02222	1.24 × 10 ⁻⁰⁵	<7.6 × 10 ¹¹	<0.5
		190.34	0.00706	8.21 × 10 ⁻⁰⁶		
Co	MET	181.42	0.01072	5.57 × 10 ⁻⁰⁶	<1.5 × 10 ¹²	<1.0
		182.24	0.02234	1.36 × 10 ⁻⁰⁵		
Zn	HTV	145.76	0.02897	2.09 × 10 ⁻⁰⁷	<4.0 × 10 ¹²	<3.1
		158.96	0.12190	3.77 × 10 ⁻⁰⁶		
As	MET	188.20	0.00250	2.24 × 10 ⁻⁰⁶	<2.0 × 10 ¹⁰	<0.02
		189.04	0.79057	8.67 × 10 ⁻⁰⁴		
Au	MET	169.93	0.05000	6.87 × 10 ⁻⁰⁶	<6.8 × 10 ¹¹	<1.6
		187.98	0.02506	2.18 × 10 ⁻⁰⁵		
Hg	LTV	184.95	1.15080	6.29 × 10 ⁻⁰⁴	5.0 × 10 ¹¹ ± 2.9 × 10 ⁸	1.2
H ₂	LTV	110–170	N/A	N/A	5.8 × 10 ¹³ ± 1.0 × 10 ¹¹	1.4
CO	LTV	139–181	N/A	N/A	1.7 × 10 ¹³ ± 1.5 × 10 ¹¹	5.7

*Estimated weight percent of element thermally desorbed in atomic form (plus H₂ and CO), assuming that 10,000 kg of soil was impact-heated to a temperature of 1000 K.

find here, based on gradients measured in Apollo samples (19).

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9. The FUV sky background seen by the LAMP is dominated by Lyα emissions from the interplanetary medium (IPM), with a much smaller amount due to UV-bright stars. The IPM emissions result when solar Lyα is resonantly scattered by the interstellar wind of H atoms blowing through the solar system, and they vary by a factor of ~2 depending on look direction. The LAMP observations in support of the LCROSS impact were directed nearly upwind, where the IPM emissions are generally brighter.

At the time of the impact, the IPM brightness was ~800 rayleighs.
10. The H₂ fluorescence spectrum was computed at 1 pm resolution before convolution with the instrumental line-spread function. Excitations from the ground state into the excited Lyman and Werner states and subsequent reemission to discrete levels and continuum were computed using the Einstein coefficients from the Atomic and Molecular database MOLAT, available at <http://molat.obspm.fr> (20–22). The H₂ fluorescence model required a high-spectral-resolution solar spectrum in order to accurately capture all important accidental resonances that contributed to the observed spectrum. We used the quiet Sun spectrum of (23), but scaled to low-spectral-resolution fluxes measured during the day of the impact acquired by TIMED-SEE and available at http://laspl.colorado.edu/see/see_data.html. For the CO fluorescence spectrum, we used the Fourth Positive (A-X) band head scattering rates (*g*-factors) as provided in Table 2 of (24), convolved with the instrumental line-spread function; these *g*-factors were recalculated for 1 astronomical unit, zero heliocentric velocity, and a rotational temperature of 1000 K.
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12. Line transition information was gathered from the online Kurucz Atomic Line Database (at www.cfa.harvard.edu/amp/ampdata/kurucz23/sekur.html) and used along with a model solar spectrum to estimate *g*-factors for each transition. The spectrum for each element was smoothed to the full width at half maximum (FWHM) instrumental resolution of ~2.8 nm [the on-orbit filled slit spectral resolution is somewhat better than the FWHM of 3.6 nm measured during ground calibration (8)]. Using these atomic spectra (comprising 395 emission lines in 22 species), a least-squares fit to the data yielded line-of-sight column densities for each element.
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26. We thank the LRO project and other LRO instrument leads for supporting the LCROSS support observations. In particular we thank C. Tooley, D. Everett, M. Houghton, A. Bartels, R. Saylor, R. Vondrak, G. Chin, J. Keller, T. Johnson, and C. Baker for making it happen. We also thank D. Goldstein and D. Summy for useful simulations and predictions, R. Killen and D. Boice for helpful discussions, and the referees for excellent comments. The LAMP is funded by NASA, whose financial support we gratefully acknowledge. One of us (D.M.H.) also acknowledges support from NASA Lunar Science Institute grant NNA09DB31A.

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Diviner Lunar Radiometer Observations of the LCROSS Impact

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The Lunar Reconnaissance Orbiter (LRO) Diviner instrument detected a thermal emission signature 90 seconds after the Lunar Crater Observation and Sensing Satellite (LCROSS) Centaur impact and on two subsequent orbits. The impact heated a region of 30 to 200 square meters to at least 950 kelvin, providing a sustained heat source for the sublimation of up to ~300 kilograms of water ice during the 4 minutes of LCROSS post-impact observations. Diviner visible observations constrain the mass of the sunlit ejecta column to be $\sim 10^{-6}$ to 10^{-5} kilograms per square meter, which is consistent with LCROSS estimates used to derive the relative abundance of the ice within the regolith.

On 9 October 2009, the Diviner Lunar Radiometer onboard the Lunar Reconnaissance Orbiter (LRO) observed a controlled impact of the Lunar Crater Observation and Sensing Satellite (LCROSS) (1) into one of the coldest places on the Moon (2). Diviner's multispectral thermal infrared measurements of this event provide insight into energy dissipation and

cooling during a planetary impact in a region where volatiles may be cold-trapped (3, 4). We report on the effects of volatiles on the temperature behavior observed by Diviner and the LCROSS Shepherd Satellite (SSc).

Diviner is a push-broom visible and infrared radiometer with nine channels spanning wavelengths from 0.3 to 400 μm , with sensitivity to

a broad range of temperatures (5) (table S1). Daytime brightness temperatures (Fig. 1B) show that the LCROSS impact site in Cabeus crater is in persistent shadow, with a nearly isothermal surface at ~ 40 K just before impact (6). Figure 1 shows that the LCROSS impact occurred near the center of one of the coldest regions of Cabeus crater. Thermal models (2) place the LCROSS impact site's annual average temperature at 37 K, in the 99.7th percentile (by area) for the region poleward of 80°S .

The LRO orbit was modified to put the closest approach (slant range 78 km) at 90 s after impact of the launch vehicle's spent Centaur upper stage, in order to better observe the LCROSS impact while protecting the spacecraft from debris. Also, the spacecraft was rolled 81.1° to allow the Lyman Alpha Mapping Project (LAMP) to view the lunar limb above Cabeus crater. Diviner

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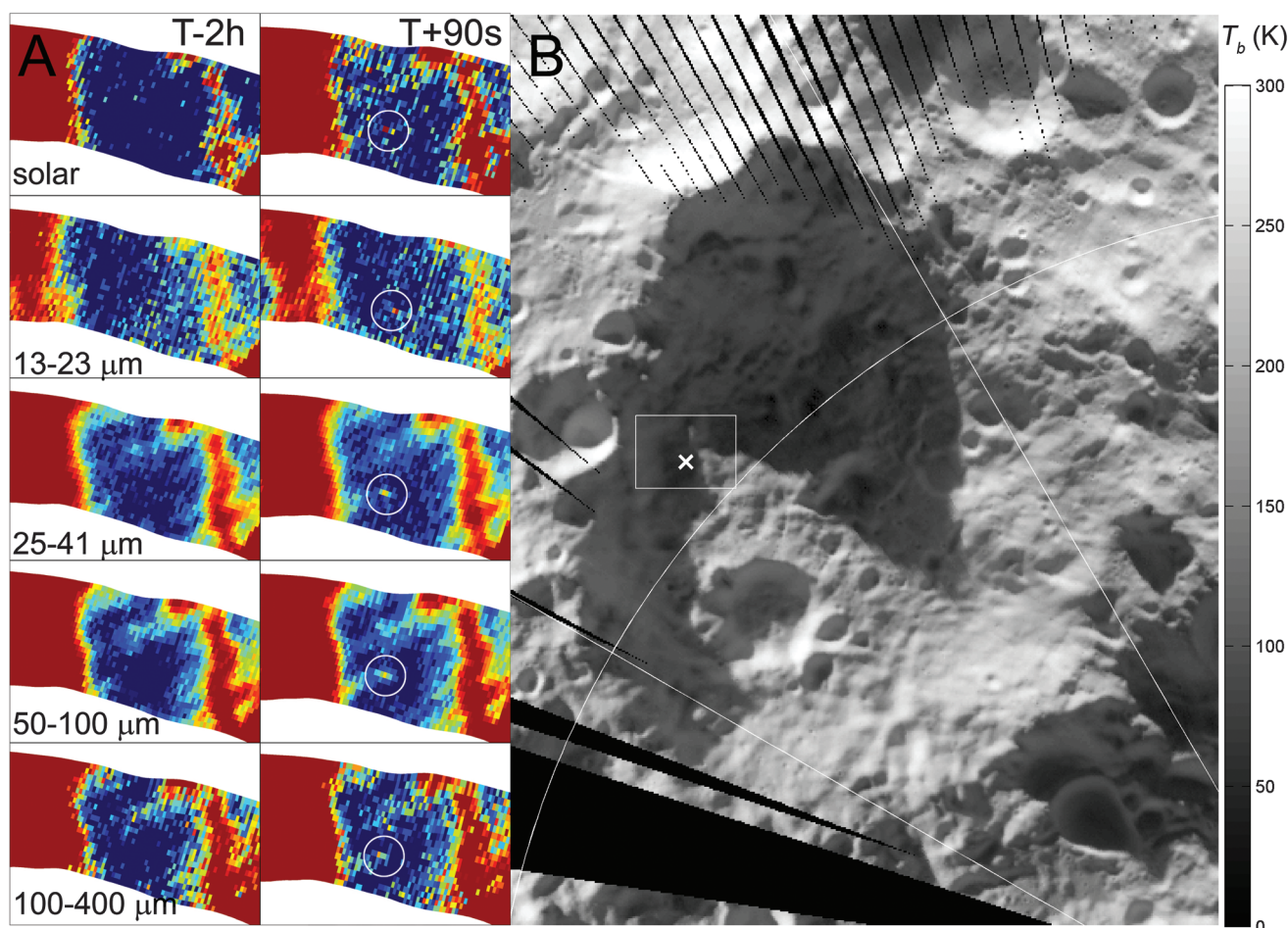


Fig. 1. (A) Pre- and post-impact images (T-2h, T+90s) of the LCROSS impact site. (B) Diviner 50- to 100- μm pre-impact brightness temperature map from nadir data, 1 to 15 October 2009. The width of each inset frame (white box) is about 15 km. The LCROSS impact site (x) had an estimated surface temperature of ~ 40 K just before impact

(6). The colors for the solar channel scale linearly with radiance from 1.2×10^{-2} to $4.3 \times 10^{-2} \text{ W m}^{-2} \text{ sr}^{-1}$; the infrared frames are linear with brightness temperature, with ranges of 85 to 105 K (13 to 23 μm), 60 to 90 K (25 to 41 μm), 55 to 80 K (50 to 100 μm), and 65 to 80 K (100 to 400 μm).

has independent azimuth and elevation actuators, enabling targeted observations despite this unusual spacecraft attitude. Diviner targeted the impact site for eight orbits, separated by about 2 hours each, the third orbit occurring about 90 s after Centaur impact (T+90s). Diviner's view of the impact site was oblique, with an emission angle of $\sim 48^\circ$ and pixel size of ~ 400 m.

All seven of Diviner's infrared channels measured thermal emission from the Centaur impact crater during the T+90s observations (Fig. 1A). Also, the more sensitive of the two solar channels observed both thermal emission from the surface and scattered sunlight from the ejecta cloud. About 2 hours later, Diviner's three longest-wavelength channels again measured a thermal signal from the subpixel impact site. At 4 hours after impact (T+4h), only the channel spanning wavelengths of 25 to 41 μm measured a signal above the noise level. No signal due to the LCROSS Centaur impact was observed on later orbits. The complete set of measurements is summarized in table S1. This report focuses only on the Centaur impact, because emission from the SSc impact (which occurred after the T+90s observations and with much lower energy) has not yet been found in the data.

In addition to thermal emission from the Centaur impact site, we attribute an enhanced signal in Diviner's solar channel, evident over a broad (~ 140 km 2) region, to scattered sunlight from the ejecta plume. Using the radiance of the scattered sunlight, we calculate a total normal optical depth of 2.0×10^{-5} to 8.1×10^{-4} over this region at T+90s (7, 8). Given a typical lunar soil grain size distribution (9), the total column mass is 1.0×10^{-6} to 4.1×10^{-5} kg m $^{-2}$. Assuming an even distribution over the 140-km 2 region, the total mass above the sunlight horizon of ~ 800 m is 2600^{+3200}_{-1400} kg (10). This range is consistent with those derived from the LCROSS spectrometer data (1), although some material may have been outside Diviner's field of view.

Thermal emission from the Centaur impact site at T+90s is dominated by near-surface material rapidly heated by friction during the im-

pact (11). A simple model using two temperature components accurately reproduces the Diviner data, captures the primary features of the spectral energy distribution (figs. S2 and S4), and is generally consistent with SSc mid-infrared images of the impact site shortly after impact (12). In this model, dissipation generates a high-temperature component within the crater (diameter ~ 25 m) and a larger region with a somewhat lower temperature represents an ejecta blanket (diameter ~ 150 m).

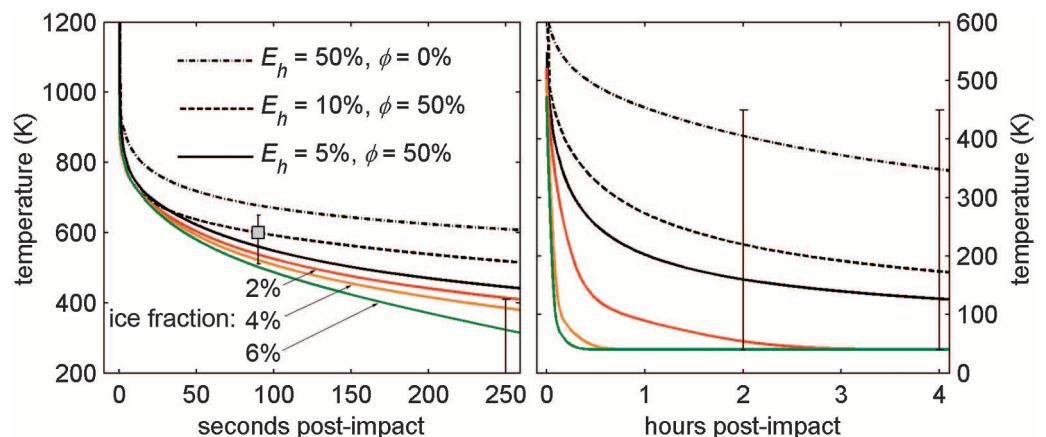
We simulated radiance values for Diviner's spectral channels (table S1) by convolving each channel's spectral response with the area-weighted blackbody spectral radiance for each of the two temperature components, with a constant background radiance filling the remainder of the field of view. Diviner's high-sensitivity solar channel cannot detect temperatures below ~ 400 K for an area less than 10^3 m 2 ($\sim 1\%$ pixel fill factor). Therefore, the clear thermal emission signal in this channel places a strong lower limit on the temperature of the hot component at T+90s. Using the 8- μm channels' T+90s radiances, we find a best-fit hot component temperature of 600^{+50}_{-90} K, with an area of 60^{+140}_{-30} m 2 (6, 13).

Diviner's longer-wavelength infrared channels constrain the average ejecta blanket temperature to be 110^{+50}_{-20} K, with an area of $8.0^{+10}_{-6} \times 10^4$ m 2 at T+90s. Between ~ 0.5 and 1 km away from the impact, brightness temperatures are elevated slightly from the pre-impact background; this is attributed to emission by warm grains in the sunlit ejecta cloud. About 2 hours after the Centaur impact, the hot region had cooled below the ~ 450 K detection threshold (area 60 m 2) of the 8- μm channels. A χ^2 minimization procedure using the observed emission in two channels (25 to 41 μm and 50 to 100 μm) failed to yield a reliable best-fit temperature for the hot component; however, we estimate a mean temperature of ~ 100 K for the combined crater-ejecta blanket region. At T+4h, the 25- to 41- μm radiance was still above the background level, but the signal was too low to provide a compelling lower limit on temperature for either component.

Cooling of the impact zone occurred primarily by surface radiation to space, sublimation of ice, and (to a lesser degree) downward conduction at the base of the hot layer. We simulated these processes with a one-dimensional heat diffusion model, including the conductivity effects of ice as well as sublimation and vapor diffusion (14). An unknown fraction of the $\sim 7 \times 10^9$ J total impact energy is irreversibly converted to heat, although an upper limit of 20% is plausible (15, 16). We investigated the cooling behavior of the hot inner region (30 to 200 m 2) for different values of E_h , the fraction of the total impact energy contributing to heating this zone (Fig. 2). Only cooling curves with $E_h > 3\%$ and initial temperature > 950 K are consistent with the T+90s observations; upper limits on these quantities are not well constrained (6). Ice within the regolith causes more rapid cooling by loss of latent and sensible heat, as well as more efficient downward conduction. If the regolith pores were filled with ice (50% porosity), we find that the heat energy fraction E_h must be $> 12\%$, otherwise cooling is too rapid to match the T+90s Diviner data. Conversely, if $E_h = 5\%$, ice mass fractions must be $< 4\%$. Thus, an independent constraint on E_h would effectively place limits on the initial ice content in the impact zone.

Diviner's estimate for the area of the hot component, 30 to 200 m 2 , is somewhat less than the area of the Centaur's impact crater, ~ 500 to 700 m 2 (12), which indicates that some portion of this region cooled below the detection limit of ~ 300 K (for an area of 500 m 2) of the Diviner 8- μm channels in less than 90 s. This is consistent with SSc NIR2 images of the Centaur crater about 4 min after impact, which reveal a dark inner region, perhaps due to more rapid cooling of icy material at depth (12). An increase in ice content of just a few percent by mass over the excavation depth (~ 1 to 3 m) could result in more than 100 K of cooling within the crater relative to the rim after 250 s (Fig. 2), in which case the crater floor should appear darker in emission. A conservative upper limit on the crater floor temperature of 410 K at 250 s after

Fig. 2. Modeled cooling curves for a 60-m 2 region of porosity ϕ heated by a fraction E_h of the total impact kinetic energy. The initial temperature is a free parameter, as is the ice content in the regolith, both of which determine the initial depth of heating (typically a few millimeters) for a given value of E_h . The data point at 90 s after impact is from Diviner data; the upper limit of 410 K at 250 s after impact refers to the dark crater observed by the LCROSS SSc (12, 17). Upper limits at T+2h and T+4h are based on Diviner thermal observations. If ice content increases with depth, interior parts of the crater should follow the steepest cooling curves. Note that the ice mass fraction (plotted for $E_h = 5\%$) can only be constrained for a given value of E_h .



impact can be derived from the relative brightness of the surrounding terrain observed by both NIR2 and Diviner (17). Model cooling curves for $E_h = 5\%$ (Fig. 2) show that a regolith ice mass fraction of $>2\%$ is required to reproduce this behavior for the assumed regolith properties.

Finally, a layer a few millimeters thick heated to an initial temperature of ~ 1000 K is consistent with both the Diviner data and the dynamics of the hydrogen vapor cloud observed by LAMP (18). At these temperatures, the regolith is desiccated nearly instantaneously, but a more gradual flux of sublimed water molecules continues as the thermal wave propagates downward. The above models imply that this gradual process accounts for $\sim 30\%$ of water molecules sublimed within the impact zone over the course of 4 min. For a heated area of 30 to 200 m^2 and ice mass fractions of 1%, 10%, and 22% (filled pores), instantaneous and gradual sublimation produce a total of 3 to 20 kg, 20 to 130 kg, and 50 to 300 kg of water vapor, respectively. This range is comparable to the ~ 155 kg of water vapor observed by LCROSS during this period (1). Thus, a substantial portion of the observed water vapor may have been supplied by the steaming crater.

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7. Optical depth was calculated from the difference in counts between the T+90s orbit and the average of the T-2h and T+2h orbits (6), converted to radiance using a factor of $3.9 (\pm 10\%) \times 10^{-3} \text{ W m}^{-2} \text{ sr}^{-1} \text{ counts}^{-1}$. Assuming single scattering, the total normal optical depth is $\tau = (4\pi\mu)/(F\varpi_0P)$, where I is the measured radiance enhancement, μ is the emission angle cosine, F is the solar irradiance within the pass band (1130 W m^{-2}), $\varpi_0 \approx 0.34$ is the single-scattering albedo, and $P \approx 0.38$ is the scattering phase function for lunar soil (8). The measurement error in the solar channel is estimated to be ~ 2 counts, giving an uncertainty of 2.8 counts ($\sim 0.01 \text{ W m}^{-2} \text{ sr}^{-1}$) in the difference.
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13. The ranges account for the combined 1σ measurement error in all channels due to instrument noise, data misregistration, background variability, and field-of-view effects (6).
14. The 1D thermal model is based on that of Vasavada *et al.* (19) and includes vertical conduction and surface emission as well as temperature-dependent thermal conductivities and heat capacities. Our ice sublimation and vapor diffusion model is based on that of Fanale and Salvail (20) for comets, where we have adapted the thermal model to adjust thermophysical properties according to local ice content. Unless otherwise

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22. Some of the research described in this paper was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA.

Supporting Online Material

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Materials and Methods

Figs. S1 to S8

Table S1

References

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Diviner Lunar Radiometer Observations of Cold Traps in the Moon's South Polar Region

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Diviner Lunar Radiometer Experiment surface-temperature maps reveal the existence of widespread surface and near-surface cryogenic regions that extend beyond the boundaries of persistent shadow. The Lunar Crater Observation and Sensing Satellite (LCROSS) struck one of the coldest of these regions, where subsurface temperatures are estimated to be 38 kelvin. Large areas of the lunar polar regions are currently cold enough to cold-trap water ice as well as a range of both more volatile and less volatile species. The diverse mixture of water and high-volatility compounds detected in the LCROSS ejecta plume is strong evidence for the impact delivery and cold-trapping of volatiles derived from primitive outer solar system bodies.

The Moon's polar regions are notable because of their potential to cryogenically trap water ice and other volatile species (1). The Lunar Reconnaissance Orbiter (2) (LRO) Diviner Lunar Radiometer Experiment has been mapping the infrared emission from the Moon

since July 2009 using seven spectral channels that span a wavelength range from 7.55 to 400 μm at a spatial resolution of ~ 200 m (3). Thermal maps of the south polar region (Fig. 1, A and B) were obtained during the LRO monthly mapping cycle just before the Lunar Crater Observation and Sensing

Satellite (LCROSS) impact (4), as the Moon approached southern summer solstice (5). The mapped quantity is the bolometric brightness temperature, which is the wavelength-integrated radiance in all seven Diviner channels expressed as the temperature of an equivalent blackbody (6). For quantifying the overall heat balance of the surface and comparing with available models, the bolometric brightness temperature is the most fundamental and interpretable measurable quantity. For the simplest case in which Diviner's surface footprint is filled with a blackbody of uniform surface temperature, the bolometric brightness temperature will be equal to the temperature of the surface. In the more general case, where Diviner's surface

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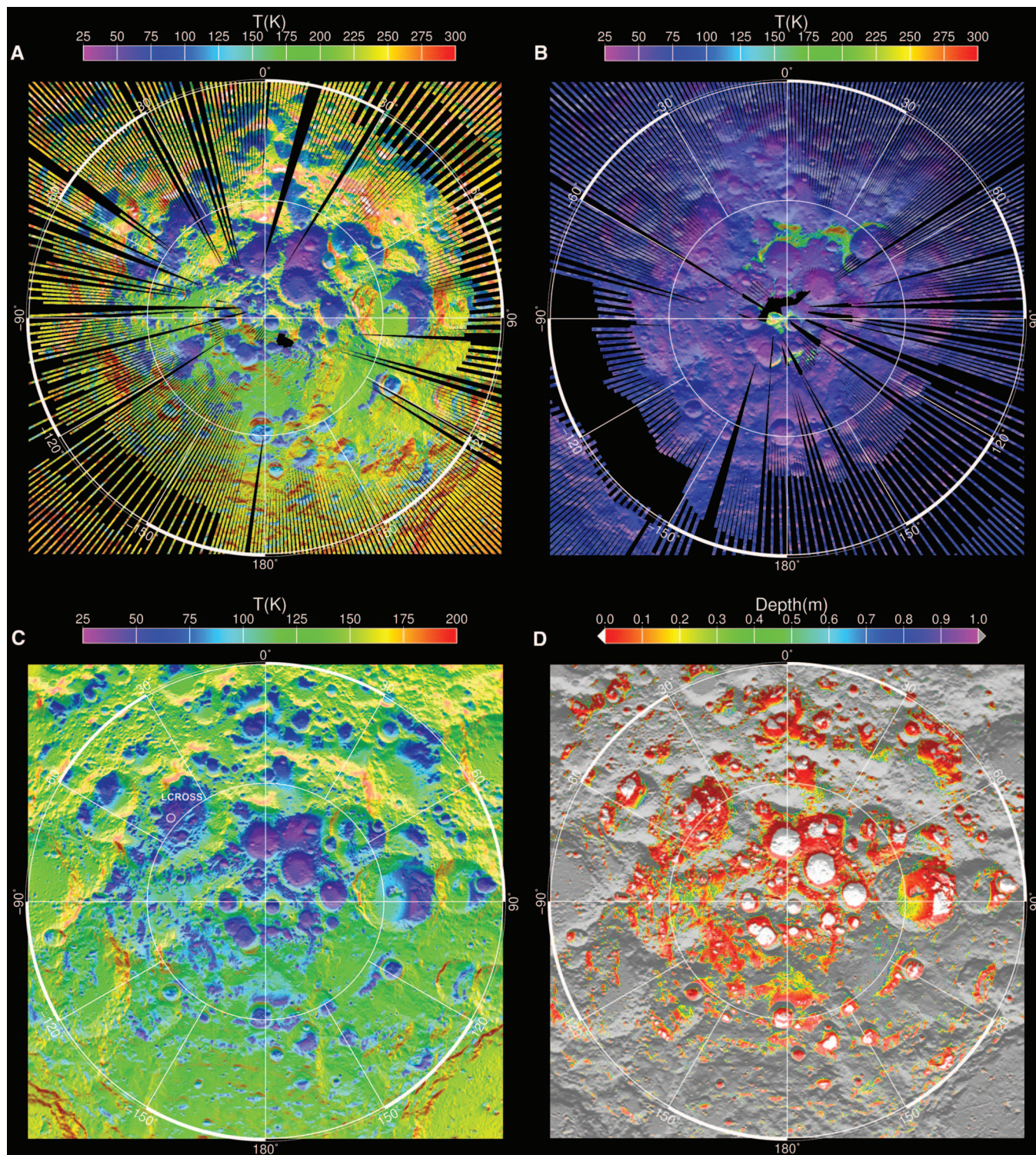


Fig. 1. Maps of measured and model-calculated surface and subsurface temperatures in the lunar south polar region. The outer circle on all maps is 80° south latitude. Observations were acquired between 6 September and 3 October 2009 as the Moon approached southern summer solstice. **(A)** Diviner-measured daytime bolometric brightness temperatures acquired between 11.4 and 13.6 hours local time (5). **(B)** Diviner-measured nighttime bolometric brightness temperatures acquired between 21.41 and 1.66 hours local time (5). **(C)** Model-calculated annual average near-

surface temperatures and the location of the LCROSS impact in Cabeus Crater. **(D)** Model-calculated depths at which water ice would be lost to sublimation at a rate of less than 1 kg m^{-2} per billion years. The white regions define the locations where water ice can currently be cold trapped on the surface, the colored regions define the upper surface of the lunar ice permafrost boundary, and the gray regions define locations where subsurface temperatures are too warm to permit the cold-trapping of water ice within 1 m of the surface. High-resolution images are available in the SOM.

footprint contains small-scale slopes, shadows, or rocks, the brightness temperatures in Diviner's individual infrared channels may vary with wavelength depending on the distribution of sub-footprint-scale temperatures, spectral emissivities, and photometric properties. In this case, the bolometric brightness temperature cannot be interpreted in terms of a unique surface temperature. However, within cold regions that are not in direct sunlight, simultaneously acquired brightness temperatures in Diviner channels 7 (25 to 41 μm), 8 (50 to 100 μm), and 9 (100 to 400 μm) are in good agreement (6), which is consistent with uniformly high spectral emissivity across this wavelength range and relatively uniform temperatures within each Diviner footprint (fig. S1, A and B). This interpretation is supported by the results of an analysis of data acquired in each of the Diviner infrared channels at the LCROSS impact site in Cabeus Crater (7). For unilluminated regions, we use Diviner bolometric brightness temperatures as reasonably accurate proxies for the temperature of the surface.

The thermal maps show that the coldest regions are located on the floors of larger impact craters that receive no direct sunlight (Fig. 1, A and B). For these regions, previous modeling studies have shown that the main heat source is emitted infrared radiation from distant interior sunlit crater walls (8–11). Topographic relief within cold crater floor regions can provide additional radiation shielding, resulting in intensely cold localized regions with measured mid-day bolometric brightness temperatures as low as 29 K. Heat flow from the lunar interior may contribute to the overall heat balance of these coldest surfaces, but is not dominant compared to heating from scattered solar and infrared radiation during this season (6).

Diviner's summer solstice observations represent a valuable snapshot of the south polar region surface temperatures that can be extended in depth and in time with models. We have developed a thermal model that realistically accounts for the effects of large-scale topographic relief on direct and indirect solar and infrared radiation on

the heat balance of the lunar surface (6). The model uses a $\sim 500\text{-m}$ -scale triangular mesh based on south polar topography derived from the Kaguya LALT laser altimeter (12) and a spatially uniform set of thermal and reflectance parameters that are generally consistent with those derived from previous studies (6). The excellent overall agreement between maps (fig. S3, A and B) and histograms (Fig. 2A) of the observed and calculated bolometric temperatures demonstrate the general validity of our modeling approach. The only notable discrepancy occurs for daytime temperatures in the shadowed portions of craters that have measured bolometric temperatures in the range of 60 to 120 K, where the model underestimates temperatures by roughly 15 K (fig. S4, A and B). This may be largely due to directionally anisotropic infrared emission from rough sunlit crater walls, which is not accounted for in the present model (6). Given the better agreement between the model and the Diviner nighttime data, we estimate that the net effect on model-calculated annual average temperatures at 2-cm depth (Figs. 1C and 2B) is less than 7 K in the warmest craters and close to zero in the coldest craters. For the limiting case of zero heat flow from the lunar interior, the temperatures at greater depths would be close to this near-surface average temperature (11). However, with non-zero heat flow, average temperatures will increase with depth at a rate proportional to the heat flow rate and inversely proportional to the thermal conductivity. Using parameters derived from the heat flow experiments at the Apollo 15 and 17 landing sites (13), we estimate that LCROSS impact site temperatures at 2-m depth should be <6 K higher than annual average surface temperatures (fig. S5). Although the Diviner bolometric temperatures presented here and cooling curves at the LCROSS impact site are generally consistent with the presence of unconsolidated regolith near the surface (7, 14), the thermophysical properties of the Moon's cryogenic regolith are not currently well constrained and could differ substantially from those in warmer regions (15), particularly at depth. We expect that the Moon's cryogenic regions extend to depths of at least tens of meters below the surface, but estimating the volumetric extent of the Moon's cryogenic regions purely from surface-temperature observations is highly uncertain.

Thermal model results can be used to estimate the stability of water ice deposits to loss by sublimation and diffusive migration through the lunar regolith (11, 16). Figure 1D shows a map of the depths at which water ice would be lost at a rate of less than 1 kg m^{-2} per billion years, which corresponds to a loss rate of 1 mm per billion years for a pure ice deposit (6). The results show that surface cold traps for water ice are surrounded by much more extensive "lunar permafrost" regions where water ice is stable in close proximity to the surface (17). These regions may receive direct solar radiation during periods when solar lighting conditions are most favorable, but maintain

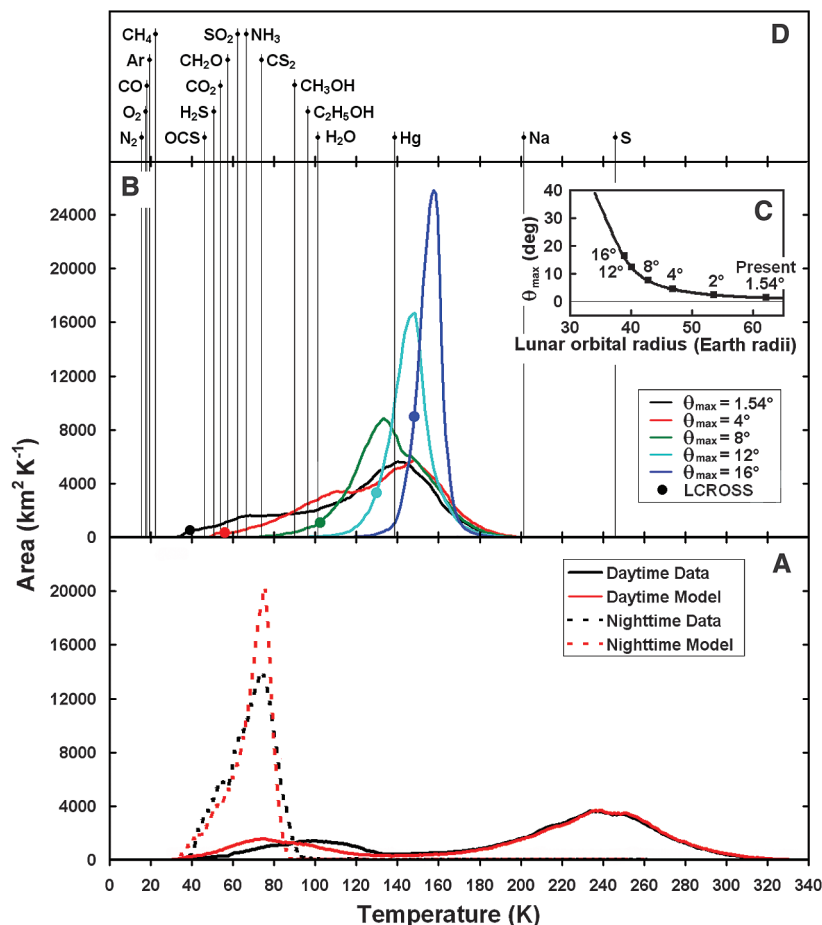


Fig. 2. (A) Normalized histograms of measured daytime and nighttime bolometric brightness temperatures for the maps shown in Fig. 1, A and B, with comparisons to model-calculated surface temperatures at the same locations and times as those of the Diviner observations (fig. S3, A and B). (B) Histograms of model-calculated annual average temperatures at 2-cm depth for the maps shown in Fig. 1C and fig. S7, A and D, and at the LCROSS impact site for selected values of θ_{max} , the mean maximum angle between the Moon's spin axis and the normal to the ecliptic plane. $\theta_{\text{max}} = 1.54^\circ$ for present-day conditions. (C) The recent evolution of θ_{max} as a function of the Earth-Moon distance (29). (D) The volatility temperatures of a range of potential cold-trapped volatile compounds (21, 22).

annual maximum temperatures at depth that are sufficiently cold to effectively prevent appreciable water loss due to sublimation. Because of their more hospitable surface thermal and illumination environments, lunar permafrost regions may be accessible locations for future in situ exploration of the Moon's cold traps.

The overall picture painted by the present thermal state of the lunar south polar region is one of extreme cold. Temperatures in the Moon's larger cold traps are closer to those expected for the poles of Pluto (18) than for Earth's closest neighbor. At the cold temperatures that exist within most south polar craters, cold-trapped water molecules have negligible mobility (16), such that any water molecules deposited on the surface will not effectively diffuse below the surface where they can be protected from loss processes such as photolysis and sputtering (19). In cryogenic regions, burial of frozen volatiles by impact gardening is likely to be a much more effective process (20). However, warmer permafrost regions that currently exist at the margins of cold traps may represent somewhat more favorable environments for the downward diffusion of water molecules into the regolith, which should be aided by the daytime temperature gradient between warmer surface layers and colder subsurface layers below.

The distribution of temperatures in the lunar south polar region also places constraints on the thermal stability of nonwater volatile species. Figure 2D shows the volatility temperatures (the temperatures at which pure solids exposed to vacuum at the surface would sublimate at a rate of 1 mm per billion years) for several volatile species (21, 22). Nonwater subsurface volatiles will also be stable to sublimation at higher temperatures owing to the effects of diffusive migration through the regolith (6). Large areas of the lunar south polar region have the capability to cold-trap water and less volatile species such as mercury and sodium. All three of these volatile species were in the LCROSS ejecta plume (4, 14, 23). Colder surface and subsurface areas in the south polar region also have the capability to cold-trap so-called super volatile species that have higher volatility than water, which include compounds such as sulfur dioxide, carbon dioxide, formaldehyde, ammonia, and methanol. The detection of a representative cross-section of these same supervolatile species in the LCROSS ejecta plume (4) represents strong evidence for the impact delivery of volatiles to the Moon by primitive outer solar system bodies, and the subsequent cold-trapping of these volatiles at the lunar poles (21, 22).

A question of interest regarding the lunar cold traps is whether they contain abundant deposits of nearly pure water ice such as those discovered by radar observations of impact craters at the poles of Mercury (24). Diviner-measured summer solstice daytime and nighttime surface bolometric brightness temperatures of 46.7 and 38.7 K in the region surrounding the LCROSS impact

site, and model-calculated annual average temperatures at this site at a depth of 2 cm, are close to 38 K (6). As shown in Figs. 1, C and D, and 2B, the LCROSS impact site is a surface cold trap for water and is among the coldest locations in the south polar region. The Lunar Prospector Neutron Spectrometer (LPNS) results show that the average hydrogen abundance in the near-surface regolith at the south pole is ~70 parts per million (ppm) by weight, which translates to a water-equivalent average abundance of ~600 ppm by weight (25). Our results show that the surface and near-surface water ice cold traps comprise >66% of the surface area poleward of 85° south latitude (Fig. 1D). If we assume that all the hydrogen detected by LPNS was uniformly distributed within these cold traps, then the average water-equivalent abundance would be only ~1000 ppm by weight, which is substantially less than the 1 to 10% water content inferred at the LCROSS impact site (4). This suggests that the LCROSS site must be enriched in water compared to the average south polar near-surface cold trap, which is consistent with enhanced hydrogen abundances observed in the Cabeus region by orbital neutron spectrometers (26–28).

The spin pole of the Moon is currently in a tidally damped Cassini State 2 configuration in which the time-averaged maximum angle between the Moon's spin axis and the normal to the ecliptic plane ($\theta_{\max}$) has decreased to its present value of $\theta_{\max} = 1.54^\circ$ as the Earth-Moon distance increased over time (Fig. 2C) (29). The absolute time scale for the tidal evolution of the Earth-Moon system is highly uncertain, but it is likely that the transition depicted in Fig. 2C has occurred over a period of more than 1 billion years (30). Model-calculated annual average near-surface temperatures $\theta_{\max} = 4^\circ, 8^\circ, 12^\circ$, and 16° (Fig. 2B and fig. S7, A to D) show that portions of the Moon's south polar region cooled considerably as the Moon's orbital radius increased, first creating cold traps capable of cold-trapping water, and then trapping compounds with higher volatility. The LCROSS impact site, which is located on the floor of the large Cabeus impact crater, is typical of the coldest areas on the Moon today. However, earlier in the Moon's orbital history, when $\theta_{\max}$ was greater than $\sim 10^\circ$, the floors of large-impact craters were not the coldest areas on the Moon because the walls of these relatively shallow craters did not shield their floors from direct solar radiation. Based on the results in Fig. 2B and fig. S7, A to D, the Moon's earliest surviving near-surface cold traps are not located on the floors of large-impact craters, but rather on the floors of intermediate-sized craters, which thus may have had longer opportunities to accumulate water ice.

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5. The LRO orbit plane is inclined 90° to the lunar equator and is fixed in inertial space. LRO's ground track rotates through 360° of longitude every sidereal month, allowing Diviner to make one daytime and one nighttime map every 27.3-day mapping cycle in pushbroom nadir mapping mode. Local time on the Moon can be expressed in hours by normalizing the angular distance between geographic longitude and the longitude of the solar point to a 24-hour day. We define daytime to be between 6 a.m. and 6 p.m. local time, and nighttime to be between 6 p.m. and 6 a.m. local time. Because the plane of the LRO orbit rotates relative to the lunar terminator by 360° every Earth year, the local times of Diviner's observations drift by ~ 2 hours during each mapping cycle. The subsolar latitude on the Moon currently varies by approximately $\pm 1.54^\circ$ over the course of the Moon's 346-day draconic year, resulting in distinct seasonal temperature variations at the highest latitudes. The LRO launch date was chosen so that the LRO orbit plane was within 10° of the noon-midnight plane during LRO's first southern summer solstice.
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Supporting Online Material

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Hydrogen Mapping of the Lunar South Pole Using the LRO Neutron Detector Experiment LEND

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Hydrogen has been inferred to occur in enhanced concentrations within permanently shadowed regions and, hence, the coldest areas of the lunar poles. The Lunar Crater Observation and Sensing Satellite (LCROSS) mission was designed to detect hydrogen-bearing volatiles directly. Neutron flux measurements of the Moon's south polar region from the Lunar Exploration Neutron Detector (LEND) on the Lunar Reconnaissance Orbiter (LRO) spacecraft were used to select the optimal impact site for LCROSS. LEND data show several regions where the epithermal neutron flux from the surface is suppressed, which is indicative of enhanced hydrogen content. These regions are not spatially coincident with permanently shadowed regions of the Moon. The LCROSS impact site inside the Cabeus crater demonstrates the highest hydrogen concentration in the lunar south polar region, corresponding to an estimated content of 0.5 to 4.0% water ice by weight, depending on the thickness of any overlying dry regolith layer. The distribution of hydrogen across the region is consistent with buried water ice from cometary impacts, hydrogen implantation from the solar wind, and/or other as yet unknown sources.

Radar reflection data returned from the Bistatic Radar Experiment conducted by the Clementine spacecraft initially suggested that deposits of pure water ice might exist in permanently shadowed regions (PSRs) near the lunar south pole (1). The lunar surface was subsequently surveyed with the Lunar Prospector Neutron Spectrometer (LPNS) (2). The energy spectrum of neutrons produced by the galactic cosmic ray particles in the uppermost layer (<1 m) of lunar soil carries the imprint of hydrogen, particularly in the epithermal energy range [see the supporting online material (SOM), section 1]. The LPNS observations from its omnidirectional neutron detectors sensors revealed a noticeable suppression of epithermal neutrons around the lunar poles above 70° latitude, which were interpreted to indicate enhancement of hydrogen and/or deposits of water ice, predominantly within PSR areas (3–5).

Here we present data from the Lunar Exploration Neutron Detector (LEND) instrument onboard the NASA Lunar Reconnaissance Orbiter (LRO) (6–9) that provide further information on the distribution of hydrogen in the lunar south pole region and thus a context for interpreting the Lunar Crater Observation and Sensing Satellite (LCROSS)

experimental results. The LEND epithermal neutron detectors are surrounded by a collimator, which provides them with a narrow field of view (FOV) corresponding to a spatial resolution of ~10 km [full width at half maximum (FWHM)] on the lunar surface from the nominal lunar orbit of 50 km [(10) and SOM, section 2]. Initial LEND data obtained from July until September 2009 were used in the selection of the target for the LCROSS impact (SOM, section 3), in conjunction with the LRO Lunar Orbiter Laser Altimeter (LOLA) altimetry data (11). The target named SP_C within Cabeus crater was selected for the final LCROSS impact (12) from among several sites (SOM, section 3), partly on the basis of the LEND neutron measurements. Here we include and discuss data obtained from 3 July 2009 to 25 July 2010. This ~1-year time interval provides for statistically robust neutron counting statistics for the lunar south polar region and allows an assessment of the following two questions: (i) What is the difference in hydrogen content at specific PSRs in comparison with that in sunlit areas at a similar latitude? (ii) How large is the difference between the observed hydrogen content at the specific PSRs in question and that in a general reference area outside of the southern polar region?

In order to estimate the hydrogen content of the regolith that is responsible for the observed suppressed neutron fluxes, we normalized the neutron count rates to a location of well-established hydrogen content. On the basis of an analysis of LPNS data, the minimum hydrogen concentration across the entire lunar surface is 10 to 50 parts per million (ppm) (3, 4). A similar value was measured in Apollo lunar soil samples (13). Therefore, we assume a minimum reference value of 10 ppm for the bulk lunar regolith hydrogen

content in the latitude belt from 60° to 70°S, just outside the extended area of polar epithermal neutron suppression. The corresponding neutron flux is at a count rate, r_0 , of about 1.9 counts per second (cps) within the instrument FOV from the sum of all four collimated LEND detectors [SOM, section 6, and (14, 15)]. All of the nine PSRs in question have surface areas (Table 1) that are larger than or comparable to the area of the LEND instrument FOV, and, as a first approximation, we therefore attribute the measured difference Δr of the count rates for them to the suppression of the neutron flux by hydrogen in the evaluated targets. In this case, the dimensionless total suppression parameter for any tested PSR site may be defined as $\xi = 1 - \Delta r/r_0$ (no suppression corresponds to $\xi = 1$, and full suppression corresponds to $\xi = 0$). The measurement error associated with this parameter can be directly computed from the statistical variability of the counts used in the estimation of Δr (Table 1) and from the uncertainty in the value of r_0 . On the basis of LEND data collected during the LRO cruise phase and model calculations, the value of r_0 could potentially be as low as 1.5 cps, as the lowest limit supported by data (SOM, section 6). We use the value of 1.9 cps for our estimates below, which will serve to bound the smallest possible value of hydrogen content and could therefore be considered to be a conservative lower limit [see also (16)].

To investigate the question (i) whether the PSRs contain significantly more hydrogen than any nearby sunlit area, we measured the difference Δr_{loc} between the epithermal neutron count rate at each of nine tested PSRs and the count rate for the sunlit (non-PSR) surface of a 360° band with the same latitude as that of the PSR (second column of Table 1) (SOM, section 4). Only two of the nine PSRs, Shoemaker (case 2 of Table 1) and Cabeus (case 3 in Table 1), show a statistically significant decrease of the neutron flux, with corresponding local suppression parameters $\xi_{\text{loc}} = (1 - \Delta r_{\text{loc}}/r_0)$ of ~0.95 and 0.85, respectively. The specific counting statistics for the seven remaining PSRs were inadequate for statistically robust measurements of their local neutron suppressions, but do facilitate the establishment of lower limits for parameters associated with local neutron suppression, as $\xi_{\text{lim}} = 1 - 3\sigma/r_0$. For example, in three specific cases (4, 5, and 7 in Table 1) they are >0.8. In two other cases [1 and 5 in Table 1 (Faustini and Haworth)], statistics permit the estimation of more conservative lower limits for the local suppression parameters, corresponding to 0.92 and 0.96, respectively. Lower limits of neutron suppression parameters ξ_{lim} between 0.96 and 0.80 correspond to relatively small upper limits for hydrogen enhancement in the evaluated PSRs of ~100 and 500 ppm, respectively (fig. S1 of SOM, section 1). The total cumulative surface area of all lunar PSRs constitutes only a few percent of the sunlit surface area within the lunar poles, and with such a small enhancement of hydrogen, they would not be expected to de-

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crease the outgoing flux of epithermal neutrons as a mechanism to explain the observed extended neutron suppression around lunar poles.

To address question (ii), we compared the average LEND count rates of the nine PSRs with the average count rate at a single southern-latitude reference belt from 60° to 70°S (third column of Table 1). This belt is located outside the polar epithermal suppression band and its average neutron flux is 5.095 ± 0.002 cps. Only three of the nine PSRs (Shoemaker, Cabeus, and Haworth) had a statistically significant decrease in their neutron flux in comparison with that of the reference belt; one PSR (Faustini, case 1 in Table 1) displays a marginally significant decrease of its neutron flux (Table 1). Site SP_C within the Cabeus PSR (case 3 in Table 1) displayed the strongest total suppression effect of about 0.33 ± 0.09 cps among all of the nine PSR targets. This result confirms the selection of this site for the actual LCROSS impact on the basis of its enhanced hydrogen content.

A contour map of the epithermal neutron flux (shown as pink in Fig. 1) delineates the neutron suppression region (NSR) within Cabeus (SOM, section 5). The difference between the outermost contour that defines the boundary of the region and the deepest inner part is ~ 0.10 cps (compare cases 10 and 11 in Table 1). It is evident that the southern part of the NSR extends beyond the PSR boundary toward the pole by up to about 20 km, and the region of greatest suppression (inner pink contour in Fig. 1) is also offset from the outline of the PSR. Furthermore, the subsurface temperature across the region with suppressed neutron emission, and not only within the PSR, is approximately 60 K, as measured by the LRO's Diviner Lunar Radiometer (17) (Fig. 1, insert). The LEND neutron data demonstrate the occurrence of local NSRs at the lunar poles, which are located both within PSRs and within sunlit regions.

The PSR within Shoemaker (case 2 in Table 1) has the largest area and a favorable south polar location for the accumulation of LEND counting statistics; thus, the total suppression parameter for this site is the most significant at 7.3σ , but its value of 0.918 ± 0.011 is still relatively small (column 4 in Table 1). The corresponding hydrogen content is estimated at 200 ppm (fig. S1 of SOM, section 1). The PSR site SP_C within Cabeus (case 3 of Table 1) displays the strongest total neutron suppression parameter, 0.825 ± 0.047 , among all of the sites, and thus the highest estimated hydrogen content of ~ 470 ppm. The average total neutron suppression parameter within the NSR associated with the entire Cabeus crater is 0.875 ± 0.023 (case 10 of Table 1), suggesting a hydrogen content of about 300 ppm. The central area of the NSR (innermost pink contour, Fig. 1), displays a suppression of 0.82 ± 0.07 (case 11 in Table 1) and thus an estimated hydrogen content of about 500 ppm. However, this value is only marginally significant, corresponding to 2.5σ with respect to the reference latitude band.

The presence of the Cabeus NSR outside the boundary of the topographically defined PSR

Table 1. Effects of the suppression of the epithermal neutron count rate within the nine PSRs from the list of LCROSS candidate impact sites and within the NSR associated with Cabeus crater (first column). Derived LEND counts for these sites are given in table S2 in section 4 of the SOM. Corresponding names and coordinates according to the LCROSS candidate impact sites are given in table S1 in section 3 of the SOM. The local neutron suppression effect is given in the second column as the difference Δr_{loc} between the average counting rate of the non-PSR (sunlit) region at the latitude of the PSR and the counting rate in the tested PSR region (see column 8 of table S2 in section 4 of the SOM). The total suppression effect is given in the third column as the difference Δr between the average counting rate in the low-hydrogen southern-latitude band at 60° to 70°S and the neutron counting rate in the tested region (see column 9 of table S2 in section 4 of the SOM). The total suppression parameter is given in the fourth column, as $\xi = 1 - \Delta r/r_0$ with respect to the reference value $r_0 = 1.9$ cps of collimated flux at a low-hydrogen region at latitudes between 60° and 70°S. Uncertainties of dimensionless suppression ξ are estimated by dividing the error of Δr by the reference value r_0 .

Name and area of region	Local neutron suppression Δr_{loc} (cps)	Total neutron suppression Δr (cps)	Total suppression parameter $\xi = 1 - \Delta r/r_0$
1. PSR Faustini (SP_A), 635 km ²	0.05 ± 0.05 (1.0 σ)	0.12 ± 0.05 (2.4 σ)	0.936 ± 0.027
2. PSR Shoemaker (SP_B), 1048 km ²	0.089 ± 0.022 (4.0 σ)	0.156 ± 0.021 (7.3 σ)	0.918 ± 0.011
3. PSR Cabeus (SP_C), 243 km ²	0.28 ± 0.09 (3.1 σ)	0.33 ± 0.09 (3.7 σ)	0.825 ± 0.047
4. PSR Cabeus B1 (SP_CB), 342 km ²	-0.01 ± 0.10 (−0.07 σ)	0.02 ± 0.10 (0.2 σ)	Not significant
5. PSR Cabeus-2 (SP_CC), 121 km ²	0.04 ± 0.14 (0.3 σ)	0.10 ± 0.14 (0.7 σ)	Not significant
6. PSR Haworth (SP_D), 973 km ²	0.012 ± 0.027 (0.4 σ)	0.08 ± 0.026 (3.0 σ)	0.958 ± 0.014
7. PSR Malapert F1 (SP_F), 264 km ²	0.01 ± 0.11 (0.1 σ)	0.05 ± 0.11 (0.5 σ)	Not significant
8. PSR Malapert-2 (SP_G), 77 km ²	-0.35 ± 0.18 (−2.0 σ)	-0.29 ± 0.18 (−1.6 σ)	Not significant
9. PSR Cabeus A1 (SP_CA), 59 km ²	0.07 ± 0.27 (0.3 σ)	0.09 ± 0.27 (0.3 σ)	Not significant
10. NSR at Cabeus, 718 km ²	—	0.24 ± 0.05 (5.3 σ)	0.875 ± 0.023
11. Center part of NSR at Cabeus, 76 km ²	—	0.34 ± 0.14 (2.5 σ)	0.82 ± 0.07

(Fig. 1) has implications for understanding the origin of enhanced hydrogen concentrations in the lunar polar regions. In sunlit areas near the lunar poles, the upper few centimeters of the soil could be heated to ~ 200 K (17). Hydrogen-rich volatiles (such as water ice) should leak out at these temperatures; thus, this surface layer should have a much smaller hydrogen content than the soil below (18, 19). The estimates of hydrogen content in Table 1 were computed assuming that hydrogen was distributed uniformly with depth (SOM, section 1), but if there is a drier (i.e., hydrogen-poor) overlying layer with 100 ppm of hydrogen, then the hydrogen content at depth will be significantly higher. For example, for a 40-, 50-, or 60-cm-thick overlying layer and for a bulk regolith density of 1.6 g/cm^3 , we would estimate that a buried soil layer in the Cabeus NSR could have a hydrogen content of ~ 600 , 1200, or 4500 ppm, respectively. The final value corresponds to a water (as ice) content of $\sim 4\%$ by weight, which is in good agreement with independent estimates of the water content asso-

ciated with the LCROSS Centaur impact site of $5.6 \pm 2.9\%$ by weight (12).

Because of the similarity of the neutron suppression and temperature in both shadowed and sunlit areas of the NSR (Fig. 1), which suggests a similar deposition of hydrogen in the shallow subsurface, a more complex model of volatile accumulation in the lunar polar regolith is required than simple cold-trapping of water molecules exclusively within PSRs. We suggest that a layer buried beneath a drier surface layer is enriched in hydrogen, and that these layers extend through both illuminated and permanently shadowed surfaces in the floor of Cabeus crater. This hypothesis is similar to that which has been considered to apply to the polar regions of Mars (20, 21). Suggested origins of the hydrogen include implantation of solar wind protons; condensation of transient water-rich atmospheres from cometary impacts, modified by gardening by micrometeorites; and space weathering (22, 23). More data is necessary from LRO to develop and test this hypothesis (24).

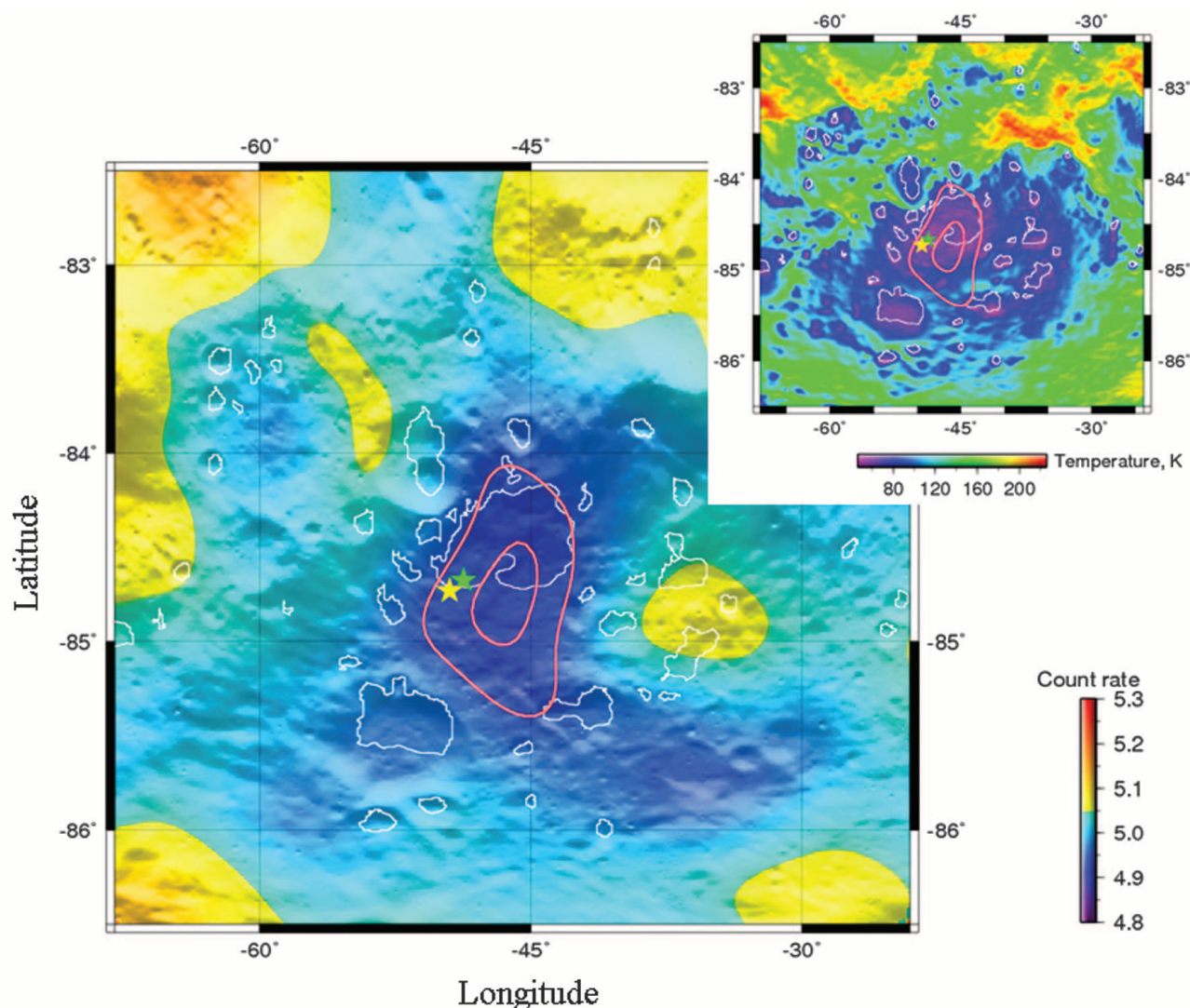


Fig. 1. LEND-derived map of the epithermal neutron flux within Cabeus crater (primary cavity). Colors represent the level of epithermal neutron flux (cps). The map is smoothed by a Gaussian filter with a 14-km scale (1σ). The thin white contours outline the boundaries of PSRs according to best-available LOLA altimetry. The outermost pink contour represents the statistically most likely boundary of the NSR within the Cabeus crater, and the innermost pink contour represents the region of strongest neutron suppression within the

NSR (cases 10 and 11 of Table 1, respectively, and SOM, section 5). The colored stars represent the locations of the LCROSS impact sites (green and yellow correspond to the Shepherding Spacecraft and the Centaur upper stage, respectively). The insert (upper right) represents the map of subsurface temperatures within Cabeus from the LRO Diviner instrument (17); the contours associated with the LOLA-defined PSRs and LEND-defined NSR are also shown on this inset temperature map (upper right).

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10. The LEND spatial resolution is a function of LRO spacecraft orbital altitude. The value of 10 km is the physical resolution of the instrument at an orbital altitude of 50 km (FWHM). This scale corresponds to an area of about 75 km² on the lunar surface. The resolution scale is inversely proportional to the orbital altitude. Over the course of the LRO mission, the altitude varied from ~30 to ~70 km (with an average of ~50 km), and the exact values at any given time are available in the Planetary Data System. The precise value of the spatial resolution is not critical to the primary conclusions of this work, as we are investigating differences in neutron count rates for PSR sites and reference regions that are larger than (or comparable to) the area of a typical LEND-resolution element or footprint (Table 1). The tested PSR target in Cabeus known as SP-C has an area of 243 km², which is larger by a factor of at least 4 than the area of a LEND-resolution element for all representative operational LRO spacecraft altitudes. Obviously, the larger the spatial resolution of the instrument surface FOV, the more difficult it will be to reliably measure contrasts in the neutron count rate. When maps are constructed from such data, the spatial resolution is also a function of the amount of spatial smoothing needed to reduce the random noise. To construct the LEND neutron counting rate map illustrated in Fig. 1, the data were Gaussian-smoothed with a spatial scale of 14 km (1σ). However, spatially smoothed data were not used for measuring the average neutron count rate for the tested targets. Additional details can be found in the SOM, section 2.
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15. LEND flight mission data support the use of 1.9 cps as the reference neutron flux, in spite of recently published results (14) that suggest much lower values on the basis of independent models of the LEND instrument and assumptions about the design of its collimator. Lower values for the neutron flux suggested in (14) would result in neutron suppression values of over 50% around the lunar poles, which are far larger than those already measured by the LPNS (2).
16. One can readily convert our estimates for the absolute suppression parameter (column 4 in Table 1) from the baseline case of $r_0 = 1.9$ cps to the lower limit case of 1.5 cps, using the expression for this parameter. In this case, the suppression curve illustrated in fig. S1 (SOM, section 1)

- could be used with new estimates of the suppression parameter to yield appreciably larger estimates of the hydrogen content in the shallow lunar subsurface.
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SOM Text
Figs. S1 to S5
Tables S1 and S2

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A New Mixing Diagnostic and Gulf Oil Spill Movement

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Chaotic advection has served as the paradigm for mixing in fluid flows with simple time dependence. Its skeletal structure is based on analysis of invariant attracting and repelling manifolds in fluid flows. Here we develop a finite-time theory for two-dimensional incompressible fluid flows with arbitrary time dependence and introduce a new mixing diagnostic based on it. Besides stretching events around attracting and repelling manifolds, this allows us to detect hyperbolic mixing zones. We used the new diagnostic to forecast the spatial location and timing of oil washing ashore in Plaquemines Parish and Grand Isle, Louisiana, and Pensacola, Florida, in May 2010 and the flow of oil toward Panama City Beach, Florida, in June 2010.

Chaotic advection theory (1–3) explains the phenomenon of mixing behavior in fluid flows with simple time dependence. This is achieved using hyperbolicity concepts associated with attracting and repelling lines, as well as hyperbolic zones with strongly chaotic behavior (4). However, chaotic advection and mixing phenomenology in that case depend on the recurrence of hyperbolic behavior and thus are difficult to extend to analyze flows with complex time dependence, such as those occurring in the ocean and atmosphere. Detecting geometric structures has become increasingly important in analyzing fluid flows with complex time dependence because of the realization that even turbulent flows feature Lagrangian structures that can be understood from the perspective of finite-time dynamical systems theory (5–7). This has led to the development of the theory of Lagrangian coherent structures (8), within which researchers focused on finite-time behavior, such as finding the attracting or repelling finite-time invariant curves (5, 6, 9, 10). In (11), an alternative approach stems from studies of the statistical properties of dynamical systems, showing that Lagrangian time-averages of physically relevant functions on state space enable the detection of invariant sets of dynamical systems, including experimentally realizable fluid flows with simple time dependence (12–14). Here we develop this theory further, based on the gradient of the average Lagrangian velocity field.

Consider the time evolution of a fluid flow on time interval $\tau = [t_0, t_0 + T]$. The dynamics of fluid particles in an incompressible two-dimensional (2D) flow on a domain $A \subset \mathbb{R}^2$ is given by

$$\dot{\mathbf{x}} = \mathbf{v}(\mathbf{x}, t) \quad (1)$$

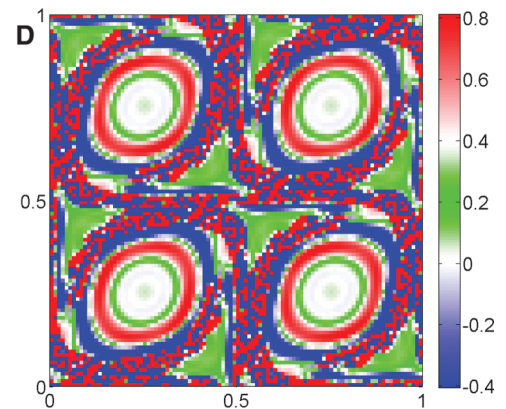
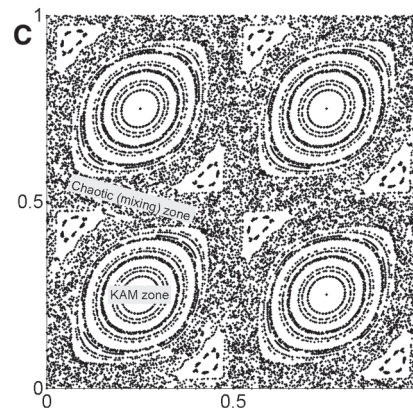
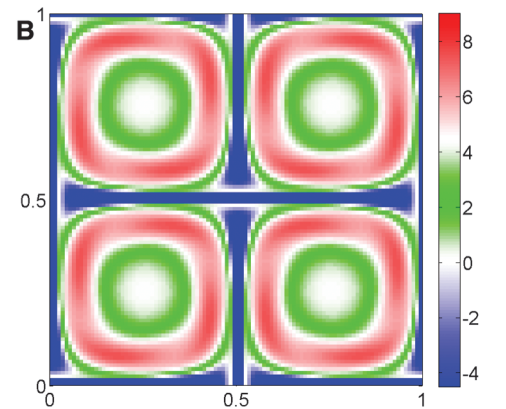
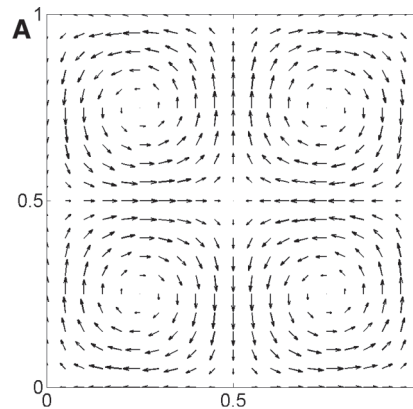


Fig. 1. (A) A cellular, divergence-free velocity field described in Eq. 6. (B) Hypergraph map for the velocity field shown in (A), for $T = 0.94248$. (C) Poincaré map for the time-periodic, divergence-free perturbation of the velocity field shown in (A) by a vector field described in Eq. 7, with $\varepsilon = 0.1$. (D) Hypergraph map for the time-periodic velocity field whose Poincaré map is shown in (C), for $T = \pi$.

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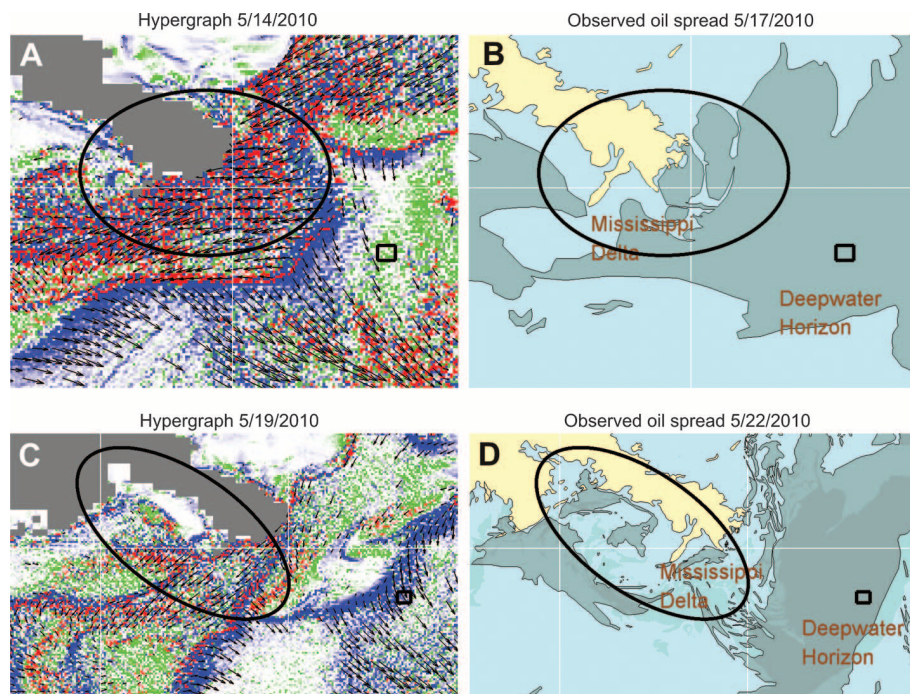


Fig. 2. (A) Ocean hypergraph map around the Mississippi Delta on 14 May, forecasting strong mixing activity (mixture of red and blue) in the following 3 days. (B) NOAA's oil spread estimate around the Mississippi Delta on 17 May. The coastal areas affected were predicted by the hypergraph map on the left 3 days earlier. (C) Ocean hypergraph map around Grand Isle, Louisiana, on 19 May, forecasting strong oil incursion (circled) in the following 3 days. (D) NOAA's oil spread estimate around the Mississippi Delta on 22 May. The coastal areas around Grand Isle affected by oil spread were predicted by the hypergraph map on the left 3 days earlier.

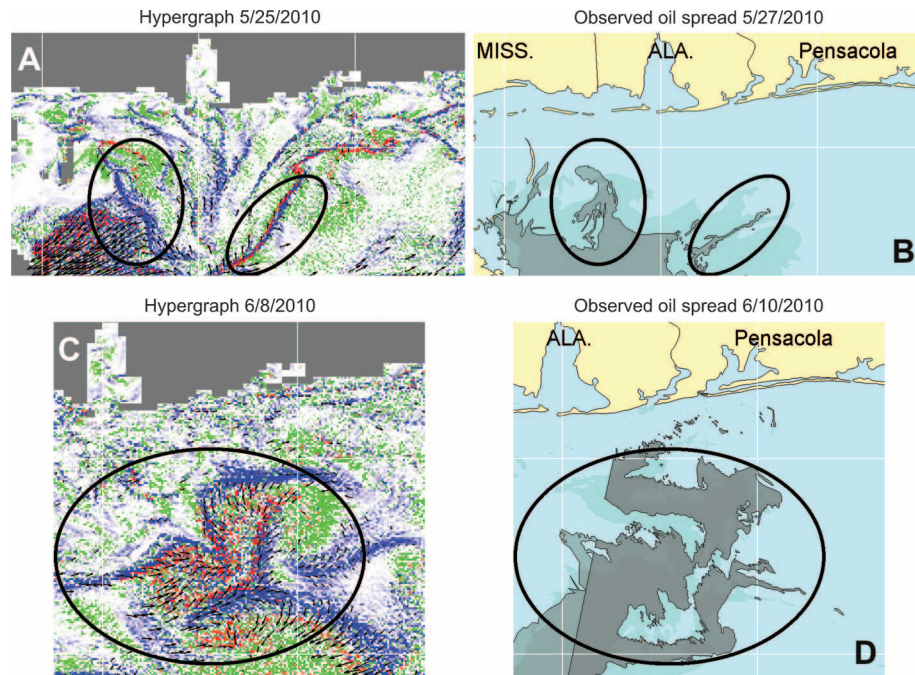


Fig. 3. (A) Ocean hypergraph map in front of the Biloxi-Pensacola shoreline on 25 May, forecasting strong oil incursion toward the coastline (circled) in the following 3 days. (B) NOAA's oil spread estimate in front of the Biloxi-Pensacola shoreline on 27 May. The major directions of oil spread were predicted by the hypergraph map 2 days earlier. The oil reached the shore several days later, on 2 June. (C) Ocean hypergraph map in front of Pensacola on 8 June, forecasting a strong oil mixing event in front of the shoreline and extension of the oil slick toward Panama City Beach in the following 3 days. (D) NOAA's oil spread estimate on 10 June in front of Pensacola. The oil developed a large slick forecasted by the hypergraph map 2 days earlier and continued to flow toward Panama City Beach.

We call $\mathbf{v}^*(\mathbf{x}_0, t_0, T)$ the mesochronic velocity field (15).

We denote by $\phi_{t_0}^{t_0+T}(\mathbf{x}_0)$ as the map of A mapping the fluid particle starting at time t_0 at point $\mathbf{x}_0 \in R^2$ to its position $\mathbf{x}$ at time $t_0 + T$. This map represents the solution of Eq. 1. Its derivative $D\phi_{t_0}^{t_0+T}(\mathbf{x}_0)$ is the Jacobian matrix $J(\mathbf{x}_0) = \partial \mathbf{x} / \partial \mathbf{x}_0$. Because $\mathbf{v}$ is divergence-free, the eigenvalues $\lambda_{1,2}(\mathbf{x}_0)$ of $J(\mathbf{x}_0)$ satisfy $\det(J(\mathbf{x}_0)) = \lambda_1(\mathbf{x}_0)\lambda_2(\mathbf{x}_0) = 1$. Thus, they are either real with $\lambda_1(\mathbf{x}_0) = 1/\lambda_2(\mathbf{x}_0)$ or complex-conjugate on the unit circle, $|\lambda_{1,2}(\mathbf{x}_0)| = 1$. We call a trajectory starting at $\mathbf{x}_0$ mesohyperbolic (hyperbolic on average) if $\lambda_{1,2}(\mathbf{x}_0)$ are real and different from 1, and mesoelliptic (elliptic on average) if the eigenvalues are complex-conjugate.

The calculation shown in (4) now leads to the conclusion that a trajectory starting at $\mathbf{x}_0$ is mesohyperbolic on τ , provided that $\det \nabla \mathbf{v}^*(\mathbf{x}_0) < 0$ or $\det \nabla \mathbf{v}^*(\mathbf{x}_0) > 4/T^2$, whereas it is mesoelliptic, provided that $0 < \det \nabla \mathbf{v}^*(\mathbf{x}_0) < 4/T^2$. There are also differences in behavior between the case $\det \nabla \mathbf{v}^*(\mathbf{x}_0) < 0$ or $\det \nabla \mathbf{v}^*(\mathbf{x}_0) > 4/T^2$. The local, linearized map behavior in the case $\det \nabla \mathbf{v}^*(\mathbf{x}_0) < 0$ is a pure strain (fig. S4A), whereas in the case of $\det \nabla \mathbf{v}^*(\mathbf{x}_0) > 4/T^2$, it is strain combined with a 180° rotation (that is, reflection across the x and y axes) (fig. S4B). When T goes to zero, the mesohyperbolicity/mesoellipticity criterion goes to the well-known Okubo-Weiss criterion (16, 17) for instantaneous snapshots of time-dependent velocity fields, where a region is called elliptic provided that $\det \nabla \mathbf{v} > 0$ in that region and hyperbolic in the region where $\det \nabla \mathbf{v} < 0$.

The Lagrangian coherent structures theory is based on the calculation of the ridges of the finite-time Lyapunov exponent (FTLE) field (7, 18, 19). In contrast to the theory of Lagrangian coherent structures that determines the stretching skeleton of a fluid flow depending on the extrema of the FTLE field [or the extrema of $\det \nabla \mathbf{v}^*(\mathbf{x}_0, t, t_0)$ over a time interval $[t_0, t]$ (20)], our approach is putting emphasis on the average behavior of trajectories over an interval of time. In contrast to the FTLE method, the mesohyperbolicity calculation distinguishes between two different regions of hyperbolic behavior (which we show in examples below enables characterization of mixing regions) and provides the ability for gradation of the elliptic regions. For more detailed comparison, see (4).

Although the field we use to distinguish kinematically separate regions is not frame-invariant, it can be improved to account for the rate of rotation of the strain along the lines pursued in (21, 22).

The field $\det \nabla \mathbf{v}^*(\mathbf{x}_0)$ becomes the centerpiece of our finite-time diagnostics of the Lagrangian properties. To build intuition, we begin with a simple, well-understood, cellular velocity field shown in Fig. 1A, described by

$$\mathbf{u}(\mathbf{x}) = \begin{bmatrix} -\sin(2\pi x_1)\cos(2\pi x_2) \\ \cos(2\pi x_1)\sin(2\pi x_2) \end{bmatrix} \quad (6)$$

This divergence-free flow has families of periodic orbits around elliptic fixed points bounded by heteroclinic orbits that connect hyperbolic

(saddle) points. In Fig. 1B, we show the $\det \nabla \mathbf{v}^*$ field for the velocity field in Fig. 1A, computed for $T = 0.94248$. The color scheme is as follows: blue indicates regions of $\det \nabla \mathbf{v}^* < 0$ (mesohyperbolic with pure strain); white and green indicate $0 \leq \det \nabla \mathbf{v}^* \leq 4/T^2$; and red indicates $4/T^2 < \det \nabla \mathbf{v}^*$ (mesohyperbolic with strain and 180° rotation). As expected, the areas around the elliptic fixed points inside the cells are white (because the period of calculation is close to the period of the linear rotation around the elliptic fixed point, over time T , particles close to the elliptic fixed point make one full circle, and their rotation angle is close to zero) and green, indicating mesoelliptic behavior there. There is a red ring of hyperbolic behavior inside the cell, due to finite-time strain and 180° rotation (this feature is also observed inside the eddies produced by the model of ocean flow in the Gulf of Mexico). The area around heteroclinic orbits is blue, indicating pure strain behavior. Thus, in this integrable case, the $\det \nabla \mathbf{v}^*$ field represents the known properties of the dynamics well.

Next, we analyze the same base field $\mathbf{u}$ perturbed by a time-dependent perturbation velocity

$$\mathbf{u}_p(\mathbf{x}) = \varepsilon \cdot \cos(2\pi t) \times \begin{bmatrix} -\sin(2\pi(x_1 - 0.25))\cos(2\pi(x_2 - 0.25)) \\ \cos(2\pi(x_1 - 0.25))\sin(2\pi(x_2 - 0.25)) \end{bmatrix} \quad (7)$$

where $\mathbf{x} = (x_1, x_2)$, $\varepsilon = 0.1$. This velocity field is time-periodic, with period 1. The standard way to visualize such systems is the Poincaré map method. The Poincaré map for the time-periodically perturbed cellular velocity field is shown in Fig. 1C. This Poincaré map shows the well-understood mixture of chaotic behavior, as indicated by orbits filling an area of the phase space, and Kolmogorov-Arnold-Moser (KAM) orbits, surrounding elliptic fixed points and elliptic periodic orbits. In Fig. 1D we show the associated $\det \nabla \mathbf{v}^*$ field, for time interval $T = \pi$. The white and green zones are positioned in the same general area where KAM curves are present. The red ring inside the KAM zone, indicating finite-time strain with 180° rotation, appears in the time-dependent flow as well. The mixing zones in the Poincaré map plot are indicated by the mixture (pure strain plus strain with 180° rotation) of the mesohyperbolic behavior, as indicated by the mixture of red and blue at those mixing locations in Fig. 1D. The feature of chaotic dynamics that we observed for the simple time-periodic cellular flow, producing a mixture of pure strain and strain plus 180° rotation in the zones of intersection of stable and unstable manifolds, has not been observed before and is interesting in its own right. We will call the plot of the field $\det \nabla \mathbf{v}^*$ the hypergraph map.

As an example of this approach, we computed mesohyperbolicity in a numerical model of the Gulf of Mexico. The model has been configured with the Hybrid Coordinate Ocean Model (HYCOM) with $1/25^\circ$ horizontal resolution and

20 layers in the vertical. Lateral boundary conditions are taken from a $1/12^\circ$ HYCOM Atlantic model. The Gulf of Mexico system assimilates satellite sea surface height (SSH) and sea surface temperature (SST) observations and runs in near real-time. The Navy Coupled Ocean Data Assimilation (NCODA) system is used for quality control and data assimilation, which includes in situ profile assimilation in addition to the surface observations. Surface wind and heat flux forcing are provided by the 0.5° NOGAPS product. The model was initialized on 2 September 2003 and continues to the present day.

Model analysis and forecasts are compared to independent (nonassimilated) infrared frontal positions and drifter trajectories. The system shows substantial ability to simulate the major circulation features of the Gulf of Mexico, including the Loop Current Extension and associated Loop Current Eddy shedding. Cyclonic shingle eddies are also accurately simulated. The system produces a 5-day forecast that contains information about hourly velocities, temperature, and salinity.

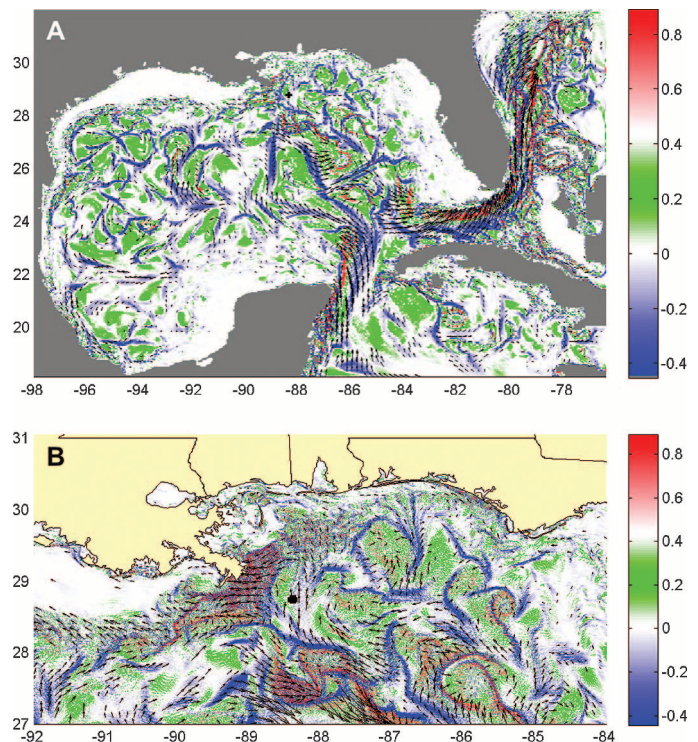
The hypergraph map is computed for a 3-day time interval starting at 0 hours [details of the computation and the model are given in (4)], using surface velocities produced by the HYCOM model. Consideration of the full physical problem would require a 3D, steady source computation of oil spill, where oil is subject to weathering and chemical reactions in the ocean. To simplify the problem, in our calculations we did not account for the differential motion between the ocean stream and the (possibly $1\text{-}\mu\text{m}$ -thick layer of) oil; that is, we treated oil as a passive scalar moved by the ocean surface velocity or alternatively as indestructible Lagrangian particles. In addition, 3D effects were

neglected. These are important near shore, but commonly enhance intense 2D mixing events (23), and thus one can view our 2D mixing predictions as an underestimate of the 3D case. The oil spill is a steady source process, whose theoretical description has received attention (24, 25), but we pursued the problem of surface transport of oil that leaked several days earlier. Thus, the constant replenishment, although important if the full problem of the oil spill is modeled, does not affect our conclusions.

Figures 2 and 3 show a sequence of hypergraph maps corresponding to the Gulf of Mexico-HYCOM model of the Gulf of Mexico surface velocity fields calculated on 14, 19, and 25 May and 8 June as compared with the estimated spread of oil several days after the hypergraph map information. We start with the estimated boundary of the oil spill at the date when the hypergraph map is calculated for the future 3 days. Isolated, elongated red and blue streaks that intersect the oil slick boundary indicate likely advancement directions of the oil slick. The oil is expected to flow in that direction, form a convergence line, and develop filaments. Diffuse zones of red and blue mixture indicate possible mixing events. Such events produce the extension of oil slicks to these zones within several days. We present several such stretching and mixing events and compare those qualitatively with available data on the oil slick extent. The oil extent was estimated by the National Oceanic and Atmospheric Administration (NOAA) (26) and is also available at the *New York Times* Web site (27).

The hypergraph map for 14 May 2010 around the Mississippi Delta (Fig. 2A) forecasted strong mixing activity (mixture of red and blue) in the

Fig. 4. (A) The hypergraph map for the full Gulf of Mexico, showing mesohyperbolic and mesoelliptic regions on 25 June 2010. This map is characteristic of the maps we computed over the 40 days of (from 10 May to 1 July 2010). (B) Zoom of the map shown in (A), showing another strong mixing event around Plaquemines Parish, Louisiana.



following 3 days. Figure 2B shows that such strong mixing events occurred, 3 days later around the Mississippi Delta and elsewhere in Plaquemines Parish, Louisiana (27). Mixing events around the Mississippi Delta continued to occur on a regular basis.

The hypergraph map (Fig. 2C) on 19 May 2010 forecasted oil incursion in the following 3 days, as was seen on Grand Isle on 22 May (Fig. 2D). The filaments seen in the data (Fig. 2D) are close to blue and red streaks in the hypergraph map (Fig. 2C).

On 25 May (Fig. 3A), the hypergraph map forecasted strong oil incursion toward the coastline between Biloxi, Mississippi, and Pensacola, Florida, in the following 3 days, along the circled convergence lines shown in red and blue. Based on this, we expected that filaments of the oil spill would extend in these directions. Such filaments were seen on 27 May (Fig. 3B). These major directions of oil spread were predicted by the hypergraph map 2 days earlier. The oil reached the shore on 2 June (27).

On 8 June 2010, the hypergraph map forecasted a strong oil mixing event (Fig. 3C), as evidenced by the mixture of red and blue in the circled area, in front of the shoreline and the extension of the oil slick toward Panama City Beach, Florida, in the following days. On 10 June 2010, a large slick developed in the area of the mixing event featured in the hypergraph map 2 days earlier, and it continued to flow toward Panama City Beach (Fig. 3D).

Based on the simulation for the full Gulf of Mexico region (Fig. 4A; the extent is typical for the situation on other dates), we estimated that the spill, if it continued, would eventually reach 80% of the Gulf of Mexico. This is an indication of what a future spill might cause if it is not contained rapidly and does not evaporate quickly. The zoom in Fig. 4B shows the situation close to the oil spill location area around Plaquemines Parish, which continued to show strong mixing activity.

Surface currents, 3-day average Lagrangian velocities, and hypergraph maps for the period from 10 May to 1 July 2010, for the full Gulf of Mexico, and the zoom for the region around the Deepwater Horizon spill, are animated in movies S1 to S6 (4).

There are many physical processes that control the fragmentation of the oil slick on the surface, including Langmuir circulations, that are not captured by the HYCOM model. However, the complexity of the arrangement of mesohyperbolic regions (Fig. 4A) seems to control the stretching and mixing events at large scales. This helps in understanding the fragmentation of oil slicks on the surface of the Gulf of Mexico featured in numerous satellite images and aerial photographs.

One use of these sorts of computations is in oil containment and cleanup. The mesohyperbolicity calculations can provide detailed spatial locations of mixing and concentration events days in advance. In addition, if a layer model is used, providing the velocity field at different depths,

information on possible locations of underwater plumes can be uncovered and supplied to research vessels so they can perform detailed measurements at those locations.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S4

References

Movies S1 to S6

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Oscillatory Mass Transport in Vapor-Liquid-Solid Growth of Sapphire Nanowires

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In vapor-liquid-solid (VLS) growth, the liquid phase plays a pivotal role in mediating mass transport from the vapor source to the growth front of a nanowire. Such transport often takes place through the liquid phase. However, we observed by in situ transmission electron microscopy a different behavior for self-catalytic VLS growth of sapphire nanowires. The growth occurs in a layer-by-layer fashion and is accomplished by interfacial diffusion of oxygen through the ordered liquid aluminum atoms. Oscillatory growth and dissolution reactions at the top rim of the nanowires occur and supply the oxygen required to grow a new (0006) sapphire layer. A periodic modulation of the VLS triple-junction configuration accompanies these oscillatory reactions.

Vapor-liquid-solid (VLS) growth processes are widely used to grow nanowires composed of functional materials such as semiconductors (1–5), oxides (6, 7), and nitrides (8, 9). Controlled growth of nanowires to a desired size, orientation, morphology, and composition requires understanding of the atomic-level growth mechanism. Relative to established growth processes such as vapor deposition and solidification, less is known about the atomic-scale mechanisms of the reaction steps involved in VLS growth (10–13). Here, we describe the growth mechanism governing self-catalytic VLS growth of (0001) sapphire (α -Al₂O₃) nanowires, which we observed by in situ high-resolution transmission electron microscopy (HRTEM).

Sapphire nanowires were grown by first forming Al droplets on an α -Al₂O₃ single crystal by heating the crystal above 660°C in a HRTEM

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hot stage while irradiating it with a focused electron beam (14, 15) (fig. S1). Movies (25 frames/s) and high-resolution images were acquired in parallel illumination with a HRTEM (JEM-ARM 1250, JEOL) operated at 1250 kV. The electron beam–induced liquid Al is not thermodynamically stable when exposed to the oxygen partial pressure in the TEM column (total base pressure of 10^{-6} Pa). At these temperatures and pressures, the most stable solid phase in the Al–oxygen binary system is α - Al_2O_3 . Once formed, the liquid Al experiences a chemical driving force to oxidize. VLS growth of α - Al_2O_3 nanowires occurred along the [0001] direction without the need to introduce a catalyst or supply an additional vapor source to the HRTEM column, so it is a self-catalytic process. The VLS growth was reproducible in a 200-kV TEM (Fig. 1A). The driving force for VLS growth is the decrease in chemical potential of Al as it transforms to stable α - Al_2O_3 . The growth kinetics, however, were observed to depend critically on the VLS triple-junction configuration (15) (fig. S4).

Al droplets formed with a broad size distribution, which resulted in the growth of α - Al_2O_3 nanowires with various diameters (Fig. 1A). The wires' length-diameter relationship is plotted in Fig. 1B, which shows that smaller droplets yield longer nanowires. This suggests that the growth rate is inversely proportional to the nanowire radius (i.e., inversely proportional to the triple-line length).

The growth of the nanowires was not prematurely interrupted by the depletion of Al;

this indicates that the liquid droplet is fed with Al during the course of the experiment. The electron beam generates not only liquid Al but also

gaseous suboxides of Al, most likely Al_2O at the temperatures and pressures of our experiments (16). The Al droplet is not passivated

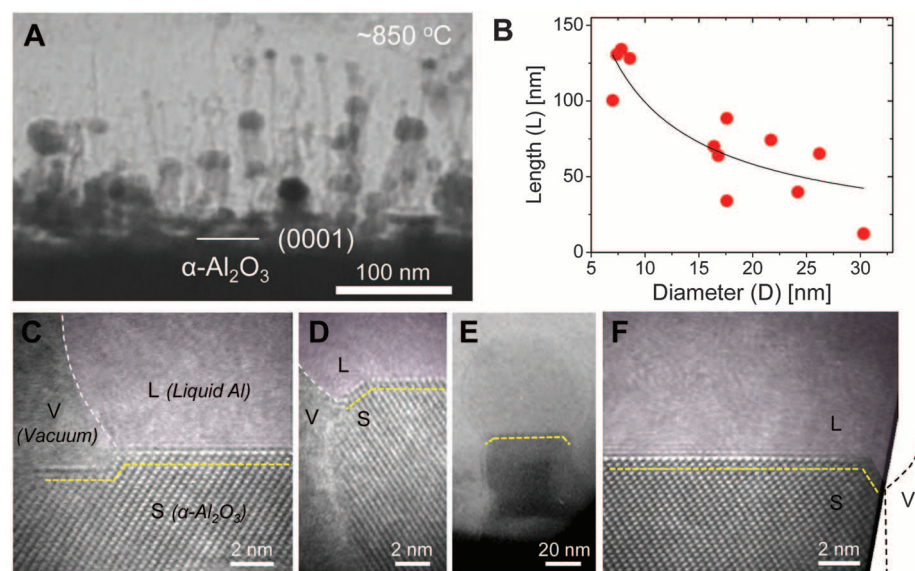
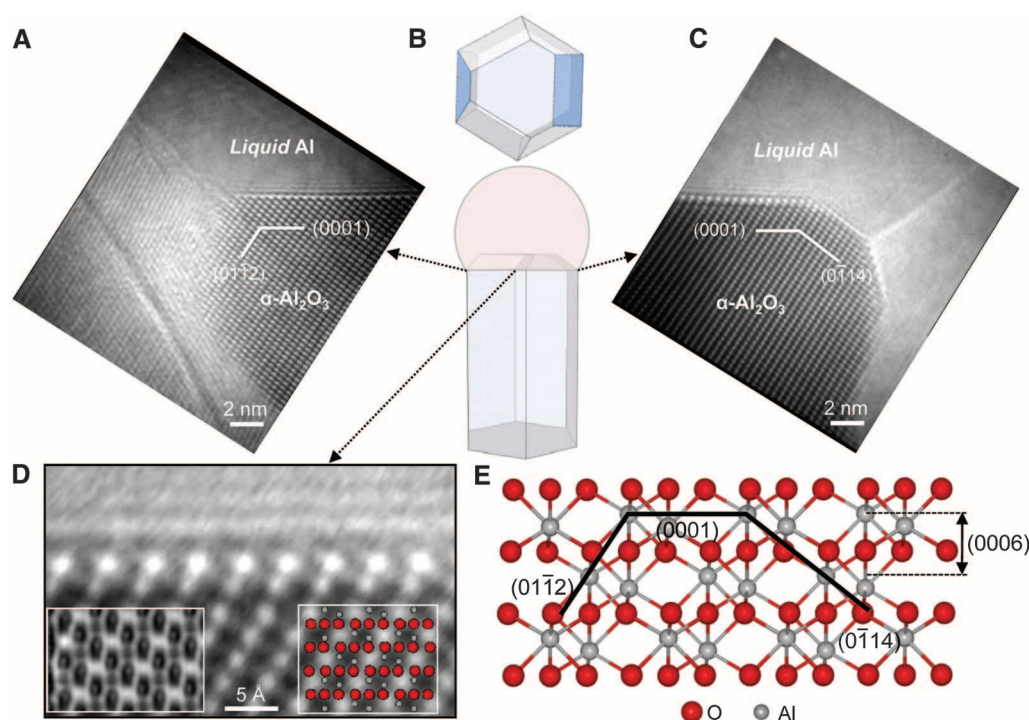


Fig. 1. Self-catalytic VLS growth of sapphire nanowires in TEM. (A) TEM image of nanowires grown at 850°C in a 200-kV TEM. Nanowires were grown epitaxially on the (0001)-oriented α - Al_2O_3 substrate with liquid Al droplets at their growth tips. The column pressure of the TEM was on the order of 10^{-5} Pa during the experiment. (B) Plot of length (L) versus diameter (D) of nanowires. The data fit correlates to the relation $L = cD^m$, where c is a constant and m is close to -1 . (C) HRTEM image showing the initial stage of nanowire growth. The nanowire growth starts with the formation and oscillation of facets at the triple phase boundary. (D) HRTEM image of the LS interface of a narrow nanowire ($D \approx 15$ nm). (E and F) TEM image (E) and HRTEM image (F) of the LS interface of a thick nanowire ($D \approx 50$ nm). Droplet edges and faceted morphologies of the LS interfaces are delineated by white and yellow dotted lines, respectively.

Fig. 2. Faceted growth morphology of sapphire nanowire. The LS interface intersects the triple junction at the (0114) and the (0112) facets, which undergo oscillatory reactions during VLS growth. (A) In situ HRTEM image (captured from movie S3) of the (0112) facet. Note that the triple phase junction in (A) is overlapped with neighboring aluminum oxides. (B) Schematic model of the faceted growth morphology of a sapphire nanowire (side view). The symmetry of α - Al_2O_3 suggests the presence of six facets at the triple junction—that is, three {0114} and three {0112} facets (top view). Along the [2110] viewing direction, only the (0114) and (0112) facets are oriented in edge-on projection (highlighted in blue). (C) In situ HRTEM image (captured from movie S1) of the (0114) facet. (D) Magnified view of the LS (0001) interface with the simulated image of α - Al_2O_3 and the projected atomic positions. The experimental imaging parameters used for the simulation are defocus = -55 nm, thickness = 25 nm. (E) The orientations of facets are indicated on the atomic model of α - Al_2O_3 to highlight their atomic stacking sequences and relative angles with respect to the (0001) plane. The unit growth layer, which corresponds to the (0006) plane, is alternately



stacked by Al (gray) and oxygen (red) sublayers, whereas the (0114) and (0112) planes mainly consist of the sublayers containing both Al and oxygen.

by a surface oxide but continuously interacts with the surrounding gaseous phases such as oxygen and Al_2O_3 , and the bulk of the droplet remains in the liquid state (15) (fig. S2). This continuous collection of oxygen and Al_2O_3 from the surrounding atmosphere and delivery to the growing nanowire acts as an infinite source, similar to standard gas-fed chemical vapor deposition processes.

All observed nanowires were found to grow along the $[0001]$ direction, regardless of their diameters, and revealed similar faceted morphologies of the liquid-solid (LS) interfaces (Fig. 1, C to F). The typical growth morphology of the $\alpha\text{-Al}_2\text{O}_3$ nanowires is shown in Fig. 2, A to D. The

major growth front corresponds to the liquid $\text{Al}\text{-}\alpha\text{-Al}_2\text{O}_3$ (0001) LS interface, which is atomically smooth and flat across the entire interface area (Fig. 2D). However, at the periphery of the LS interface near the VLS triple junction, the interface changes in orientation from (0001) to (01 $\bar{1}2$) and (01 $\bar{1}4$) facets (Fig. 2, A and C), respectively. When a nanowire is viewed in the $[2\bar{1}\bar{1}0]$ direction, only the (01 $\bar{1}4$) and (01 $\bar{1}2$) facets are visible in edge-on projection (highlighted in blue in Fig. 2B). These nanometer-wide facets preserve the concave shape of the LS interface, which is favored for the force balance with the liquid-vapor (LV) and solid-vapor (SV) surface tensions at the triple phase junction (17).

Moreover, these facets were seen to oscillate in size during the dynamic growth and dissolution reactions, which facilitates mass transport between the interfaces (movies S1 to S3). The formation of the facets and their dynamic oscillation are different in origin from those reported in (2). In the case of Au-catalytic Si nanowire growth, the facets occurred because the orientations parallel to the growth direction were not stable (2). In our case, the formation of facets at the corners of the anisotropic $\alpha\text{-Al}_2\text{O}_3$ nanowires, which are in contact with liquid Al, lowers the energy of the system, as was calculated for anisotropic particles in contact with a deformable boundary (18).

In situ HRTEM observations revealed the reaction pathway for oxygen (movies S1 to S3): Oxygen is transported to the LS interface from locally oscillating growth and dissolution reactions at the top edge of the nanowire. Oxygen is supplied to the LS (0001) interface while the facets are growing (i.e., while the top rim of the nanowire is sacrificially consumed). Oxygen from the LV surface is used to shrink the facets (growth normal to the facet planes). The reaction front for both growth and dissolution corresponded to the $\alpha\text{-Al}_2\text{O}_3$ (01 $\bar{1}4$) or (01 $\bar{1}2$) facets in all observed nanowires growing along the $[0001]$ direction.

Figure 3, A to F, shows several key images (captured from movie S1) representing an oscillation period, starting from crystal growth on the (01 $\bar{1}4$) facet. During this stage, the LV surface moves together with the (01 $\bar{1}4$) reaction front and a crystalline rim is formed (Fig. 3, A to C). Crystal growth on the (01 $\bar{1}4$) facet is facilitated by the continuous diffusion of oxygen (or Al_2O_3) from the LV surface through the triple junction (19), so that growth is driven by the oxygen partial pressure (i.e., the chemical potential of oxygen). When growth shrinks the facet to a point (expected to be shortly after Fig. 3C), the LV surface is directly connected to the LS (0001) interface. This causes a sudden change in the dihedral angles seen at the triple junction (Fig. 3I). The growth of a new $\alpha\text{-Al}_2\text{O}_3$ (0006) layer and the sacrificial dissolution of the top rim of the nanowire begins when this condition is met (Fig. 3, D to F). The sacrificial dissolution nearly restores the same triple-junction configuration [i.e., the same dihedral angles and (01 $\bar{1}4$) facet orientation] as seen in the growth stage (Fig. 3H). Dissolution stops when the supply of oxygen for a new $\alpha\text{-Al}_2\text{O}_3$ (0006) layer is met (Fig. 3F). At this point, growth on the (01 $\bar{1}4$) facet starts again, the facet begins to shrink as it is supplied with oxygen (or Al_2O_3) from the surface of the liquid droplet, and a new oscillation cycle begins. A similar oscillatory behavior is observed at the (01 $\bar{1}2$) facet at the opposite side of the nanowire, but a smaller volume is involved and therefore it is more difficult to discern (fig. S3 and movie S3). Our in situ observations were conducted in the high-vacuum environment of a TEM during high-energy electron beam irradiation (electron dose rate $\sim 10^5$ to 10^6 electrons $\text{s}^{-1} \text{nm}^{-2}$) (15). Under

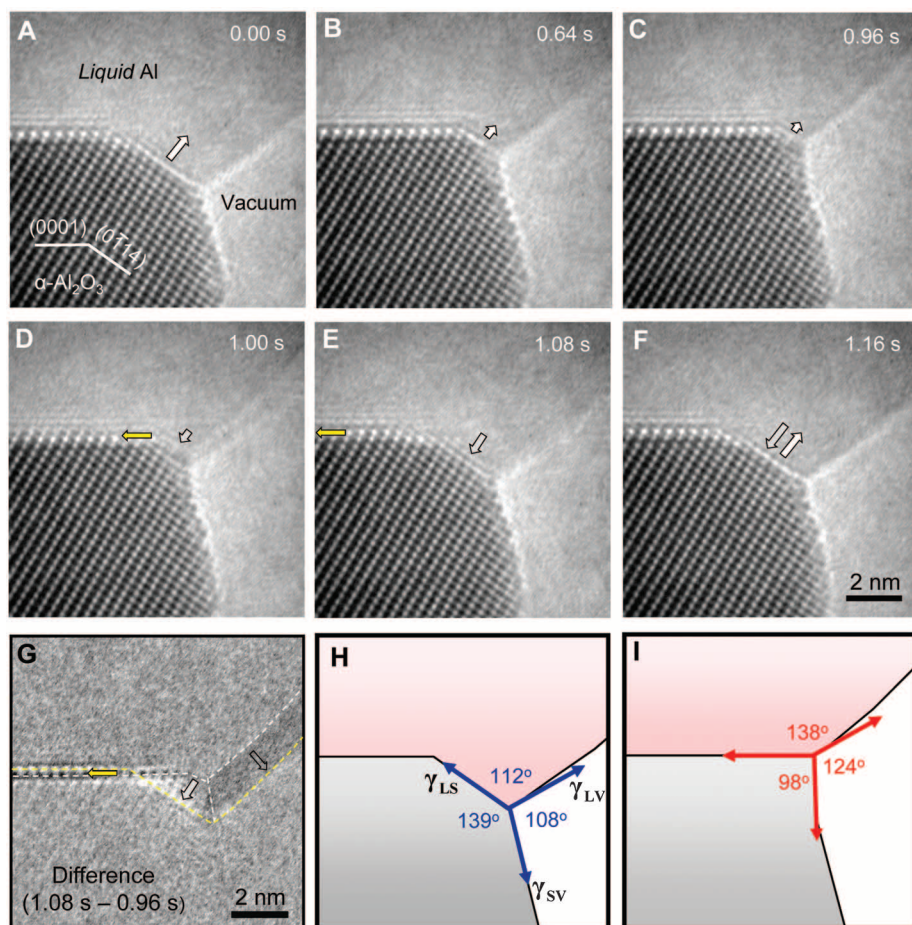


Fig. 3. Oscillatory mass transport in the VLS growth of sapphire nanowires. A series of HRTEM images outlines a period of oscillatory growth and dissolution reactions. (A to F) Images were captured from a real-time movie (movie S1) at the elapsed times shown. Local crystal growth of a rim at the triple-junction region by mass diffusion from the LV surface to the $\alpha\text{-Al}_2\text{O}_3$ (01 $\bar{1}4$) facet is shown in (A) to (C). During this process, the (01 $\bar{1}4$) facet shrinks. Shortly after (C) is reached, the LS (0001) interface comes in a direct contact with the LV interface. Dissolution of the previously grown crystalline rim on the (01 $\bar{1}4$) facet is shown in (D) to (F). Dissolution continues to supply oxygen until a complete (0006) layer is added on the interface. The end of dissolution is shown in (F). Arrows in each figure indicate local crystal growth (white), dissolution (gray), and oxygen diffusion (yellow). (G) Difference image obtained by subtracting the video image (C) from (E). The locations of LV and LS interfaces before and after dissolution are delineated by white and yellow lines, respectively. (H and I) Schematic illustration of the triple-junction configuration during local crystal growth on the (01 $\bar{1}4$) facet (H) and at the end of growth (I). Note that the dihedral angles are almost the same for growth and dissolution. The surface (and interface) tension forces (not to scale) and the measured values of dihedral angles are indicated.

different electron dose rates, oxygen partial pressures, and/or temperatures, the growth mode and configurations may be different from those we observed (20).

The nanowire growth proceeds layer-by-layer via formation of new $\alpha\text{-Al}_2\text{O}_3$ (0006) layers at the LS (0001) interface. The unit growth layer, $\alpha\text{-Al}_2\text{O}_3$ (0006), consists of stacks of alternating Al and oxygen (Fig. 2E). Comparison of the experimental and simulated HRTEM images (Fig. 2D) suggests that the LS (0001) interface most likely adopts an Al bilayer termination. This termination corresponds to one of several low-energy configurations of the $\alpha\text{-Al}_2\text{O}_3$ (0001) surface when in contact with Al (21). Adjacent to this interface, the liquid Al atoms are arranged in solid-like positions in the liquid (14). The interface-induced layering (ordering) decays exponentially with distance from the interface. The contrast modulation in the liquid adjacent to the solid (Fig. 2D) indicates some in-plane ordering, with the location of Al atoms in the liquid phase at positions extrapolated from the Al sublattice of $\alpha\text{-Al}_2\text{O}_3$.

Oxygen arriving to the interface causes relaxation of the interface structure until a new layer of $\alpha\text{-Al}_2\text{O}_3$ forms (Fig. 4). The relaxation on the $\alpha\text{-Al}_2\text{O}_3$ side (marked by a black asterisk) may be due to the adsorbing oxygen, which disturbs the stable Al termination of the $\alpha\text{-Al}_2\text{O}_3$ lattice (21). Within a very short time (~ 0.12 s), the stable Al termination is restored upon growth of a new (0006) layer. The relaxation in the liquid atom ordering (marked by a white asterisk) is related to the nonuniform layering distances during growth (22, 23). As Al atoms in the first ordered liquid layer (~ 1.6 Å from the interface) are incorporated into the $\alpha\text{-Al}_2\text{O}_3$ lattice together with the adsorbed oxygen, those in the second Al layer (~ 2.5 Å from the first ordered layer) are pulled toward the new interface to reach stable positions.

The growth of the $\alpha\text{-Al}_2\text{O}_3$ (0006) layers is limited by oxygen diffusion to the interface. Direct diffusion through the liquid Al is one possible route. However, this is unlikely because the amount of oxygen that can be dissolved in liquid Al is limited by the extremely low solubility (at 1000°C the solubility is 0.3 ppm) (16, 24). Thus, the triple junction is the only access point for the oxygen adsorbed on the surface of a liquid droplet to diffuse to the LS interface.

Dissolution of the top rim of the nanowire provides the oxygen required for growth of a (0006) layer under the low oxygen pressure in the microscope column. A simple calculation confirms that the number of oxygen atoms released by dissolution during formation of the facets is roughly the same as the number required to form a single $\alpha\text{-Al}_2\text{O}_3$ (0006) layer (25). The oscillatory ($0\bar{1}14$) facets appear to be the primary oxygen source, supplying about two-thirds of the oxygen needed for the new layer, and the rest is supplied by the ($01\bar{1}2$) facets at the opposite triple junction. Oxygen is supplied to the LS interface from the multiple facets through their in-phase

oscillatory behavior at the top rim of the nanowire. Time-frame analysis of the oscillating ($01\bar{1}2$) facet gives an oscillation frequency of $\sim 1.0\text{ s}^{-1}$ (fig. S3), which is slightly higher than that of the ($0\bar{1}14$) facet, 0.9 s^{-1} , but in reasonable agreement within the limited time resolution. Note that the time for dissolution, measured to be 0.16 s, is identical for both ($0\bar{1}14$) (Fig. 3) and ($01\bar{1}2$) facets (fig. S3); this suggests that both facets form and disappear in phase and supply oxygen to the LS interface.

These observations show that the triple-line region plays an important role in the self-catalytic growth of (0001)-oriented Al_2O_3 nanowires. For systems with a high solubility of the growing species within the liquid, the influence of the triple junction is often neglected, and constant contact angles are assumed (26, 27) or the processes at the triple junctions are related to nonstable growth orientations (2, 27). In our case, we observe that the contact angles change periodically. They appear to be fixed while the facets are forming and shrinking, but they are different when the LV surface contacts the (0001) solid surface. At this point, the nanowires start to form a new (0006) layer. There is no gradient in the oxygen chemical potential at the LS (0001) interface until this stage is reached. The interface energy between $\alpha\text{-Al}_2\text{O}_3$ (0001) and liquid Al is reduced by oxygen adsorption (24), presumably to the region of ordered liquid Al. Oxygen for the new layer comes from

the outer, top rim of the nanowires. If the supply of oxygen from the microscope column is limited, it is the reduction of the LS (0001) interface energy that forms the driving force for dissolution of $\alpha\text{-Al}_2\text{O}_3$ and formation of the facets at the top rim of the nanowire. At the same time, arrival of oxygen (Al_2O) from the surface of the liquid droplet and the microscope column facilitates growth normal to the facets and their eventual eradication. Growth normal to the facets is slow relative to growth of the nanowire in the [0001] direction. Hence, this is the rate-limiting step in this cyclic process. When the facets are completely eradicated, the LV surface is once again in contact with the LS (0001) interface and the process is repeated.

As discussed by Schwarz and Tersoff (27), the forces present at the triple junction are important for the nucleation of a new layer. When the LV surface is in contact with the LS surface, the force normal to the LS interface is reduced. This reduces the chemical potential of $\alpha\text{-Al}_2\text{O}_3$ at the triple junction and allows the formation of a critical-size nucleus. Once this nucleus forms, two different growth scenarios can be envisioned. In the first scenario, we speculate that the inner step of the new layer moves toward the center of the nanowire by incorporating oxygen, supplied by the dissolving outer rim of the nanowire, to kink sites on the inner edge of the growing layer. The driving force for this process is the

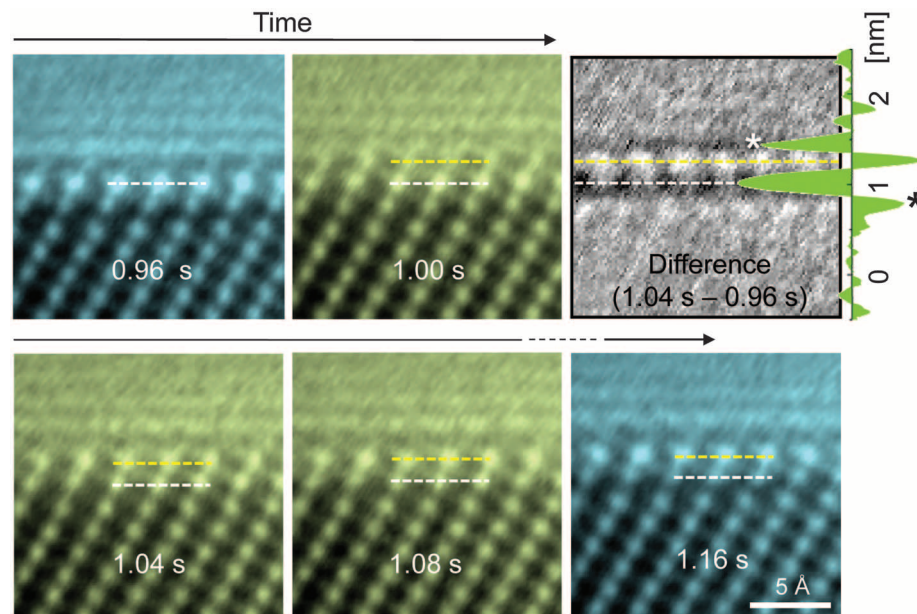


Fig. 4. Layer-by-layer growth of a (0006) layer. Magnified views of the video frame images show structural evolutions of the LS (0001) interface with the onset of oxygen adsorption. Oxygen diffusion and adsorption into the interface, particularly between the ordered framework of liquid Al atoms and the Al-terminated LS (0001) interface, cause relaxation of interface structure until a new (0006) layer of $\alpha\text{-Al}_2\text{O}_3$ has formed (images highlighted in yellow). The complete formation of a new (0006) layer takes 0.16 s (images highlighted in blue). The original interface and the new interface positions are delineated by white and yellow lines, respectively. The interlayer relaxations are traced in the difference image (upper right), obtained by frame-to-frame subtraction. The interlayer relaxations in the layered liquid Al and $\alpha\text{-Al}_2\text{O}_3$ subinterface layers are traced by the intensity line profile and marked by white and black asterisks, respectively.

reduction in chemical potential by forming the crystalline phase. In the second scenario, we assume that oxygen has first adsorbed to the LS interface, driven by the reduction in interface energy (24, 28), having diffused along the interface from the triple junction. Once oxygen adsorbs, a critical-size nucleus (in the form of a step) forms at the triple junction and sweeps across the LS (0001) interface, forming a new (0006) layer of $\alpha\text{-Al}_2\text{O}_3$ in its wake. Oxygen then again adsorbs to the new LS interface, supplied from the dissolving outer rim of the nanowire. The nucleation rate of steps is relatively low under the present experimental conditions, leading to single steps that sweep across the interface (no defects resulting from the intersection of steps or multiple nucleation events were detected). Both of these reactions would be very fast; the formation of a new (0006) layer after oxygen diffusion has started requires only ~ 0.16 s. Our experimental observations cannot distinguish between these two scenarios.

Our in situ HRTEM observations have revealed an oscillatory growth process that is not obvious from ex situ observations of grown nanowires. These observations provide an explanation for the (0001) growth mechanism and why it is not continuous. What distinguishes this reaction from most VLS processes is that the catalyst droplet is not directly involved in supplying all the necessary components for growth. Instead, oxygen for the new (0006) layer is supplied by transient sacrificial dissolution of the top rim of the nanowires via facet formation. The components of interface tensions at the triple junction also promote formation of the facets and probably drive the nucleation of steps of solid (0006), which sweep across the LS (0001) interface, resulting in growth of a new (0006) layer. Formation of the (0006) layer is relatively fast because the Al atoms on the liquid side of the LS surface

are already in positions similar to those in the underlying bulk sapphire. The oxygen collected on the LV surface is used to eradicate the facets at the outer, top rim of the nanowires once a complete (0006) layer is formed. It is this process that is the rate-limiting step.

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Species Selection Maintains Self-Incompatibility

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Identifying traits that affect rates of speciation and extinction and, hence, explain differences in species diversity among clades is a major goal of evolutionary biology. Detecting such traits is especially difficult when they undergo frequent transitions between states. Self-incompatibility, the ability of hermaphrodites to enforce outcrossing, is frequently lost in flowering plants, enabling self-fertilization. We show, however, that in the nightshade plant family (Solanaceae), species with functional self-incompatibility diversify at a significantly higher rate than those without it. The apparent short-term advantages of potentially self-fertilizing individuals are therefore offset by strong species selection, which favors obligate outcrossing.

The astonishing diversity of flowering plants has led to numerous hypotheses linking the propensity for diversification to morphological, life history, and physiological char-

acteristics (1–4). Although it is clear that no single character can entirely explain the variation in plant diversity (2, 4), many traits potentially associated with an increase in species richness

may have been overlooked because of methodological shortcomings (5, 6).

The overwhelming majority of flowering plants function as both males and females, but despite the potential for self-fertilization in hermaphrodites, most of these plants predominantly or exclusively mate with other individuals (outcross) (7, 8). Outcrossing is often enforced by self-incompatibility (SI): the ability of individual plants to recognize and reject their own pollen. Each SI system characterized to date relies on

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complex genetic mechanisms (9). Their value is primarily attributed to the avoidance of inbreeding depression (10), but the short-term disadvantages of SI, which limits an individual plant's ability to reproduce if outcross pollen is not available, is expected to result in frequent transitions to self-compatibility (SC) (8). Mate limitation and the automatic advantage of selfing (10, 11) each favor the spread of mutants that break down SI. Indeed, angiosperm families in which SI is found also contain significant proportions of SC species (12). The two states are commonly interspersed within clades, implying frequent evolutionary transitions. Independent data, both genetic and phylogenetic (12, 13), suggest that the transition from SI to SC is one of the most common evolutionary shifts in flowering plants (14). Studies of the genetic basis of SI indicate that novel SI systems originate very rarely (9, 15). The combined evidence implies that SI is frequently lost by species and that an identical form of SI is only rarely, if ever, regained (12).

In Solanaceae species with SI, strong negative frequency-dependent selection maintains dozens of alleles at the locus controlling self-recognition (S-locus), where it has preserved ancestral polymorphism over 40 million years (13, 16, 17). After pollination, if the haploid pollen allele matches either allele of the diploid female reproductive organ, fertilization is prevented. Individuals with common alleles therefore have fewer potential mates, and those with rare alleles more, so that alleles ordinarily lost by drift are instead maintained over long time scales (16). The outcome is a pattern of ancestral polymorphism shared not only across species boundaries but among distantly related genera. Randomly sampled alleles from different SI species of Solanaceae are often more closely related than those within species (16, 17). This provides strong evidence that a polymorphic and therefore functional S-locus was present in the common ancestor and passed on to descendant SI species in the family (17).

Although the SI mechanism has been frequently lost and not regained within Solanaceae (13), it occurs at an intermediate frequency. The proportion of SI species is declining with each transition to SC, so SI may be destined for extinction. But the SI mechanism of Solanaceae is ~90 million years old, nearly as old as eudicots (15), and SI may instead persist if the diversification rate of SI lineages exceeds that of SC lineages by at least the SI-to-SC transition rate (17). In this scenario, the loss of SI may be countered by a greater difference between speciation and extinction rates associated with the presence of SI relative to SC. A strong association between SI and increased diversification would provide unusually compelling evidence for a causal relationship, because frequent character changes to SC uncouple the evolution of the focal character from others and reduce the chance that an inferred connection is spurious (18).

We examined the effect of SI on diversification in Solanaceae. The nightshades contain

many agriculturally important species (such as tomato, potato, chile, and tobacco), so phylogenetic and breeding system data are extensive. Among the estimated 2700 species in the family, approximately 57% are SC, 41% are SI, and less than 2% have separate male and female plants (19).

We constructed a phylogeny through an initial alignment matrix of 998 species and eight genes, and we assigned character states (SI or SC) for 616 species (19). Subsequent analyses concentrated on a monophyletic clade termed “ $x = 12$ ” for its uniform base chromosome number, containing approximately 2300 species, including the subfamilies Solanoideae and Nicotianoideae (20) [we also conducted analyses on the whole family (19)]. Both character states and phylogenetic data are better sampled in this clade than in the rest of the family, yielding 356 species present in the tree and with known mating systems

(Fig. 1). This sample did not statistically differ in SI frequency from the total available data set of 616 species ($P > 0.3$), and our analyses accounted for sampling incompleteness (21).

Irreversible loss of SI in Solanaceae will cause the frequency of SI to decline to zero if $r_1 \leq r_C + q_{IC}$, where r_1 and r_C are net diversification rates (the difference between speciation and extinction rates) for SI and SC lineages, respectively, and q_{IC} is the per-lineage rate of transition from SI to SC. Alternatively, the loss of SI may be offset by more rapid diversification of SI lineages when $r_1 > r_C + q_{IC}$. In this latter case, the equilibrium proportion of SI species, p_{SI} , is positive and equal to $1 - q_{IC}/(r_1 - r_C)$ (17).

We analyzed character evolution and diversification on the maximum likelihood tree (Fig. 1) and also on 100 bootstrap trees to assess robustness to phylogenetic uncertainty (19). Rates of speciation (λ_1 and λ_C ; the subscripts denote SI

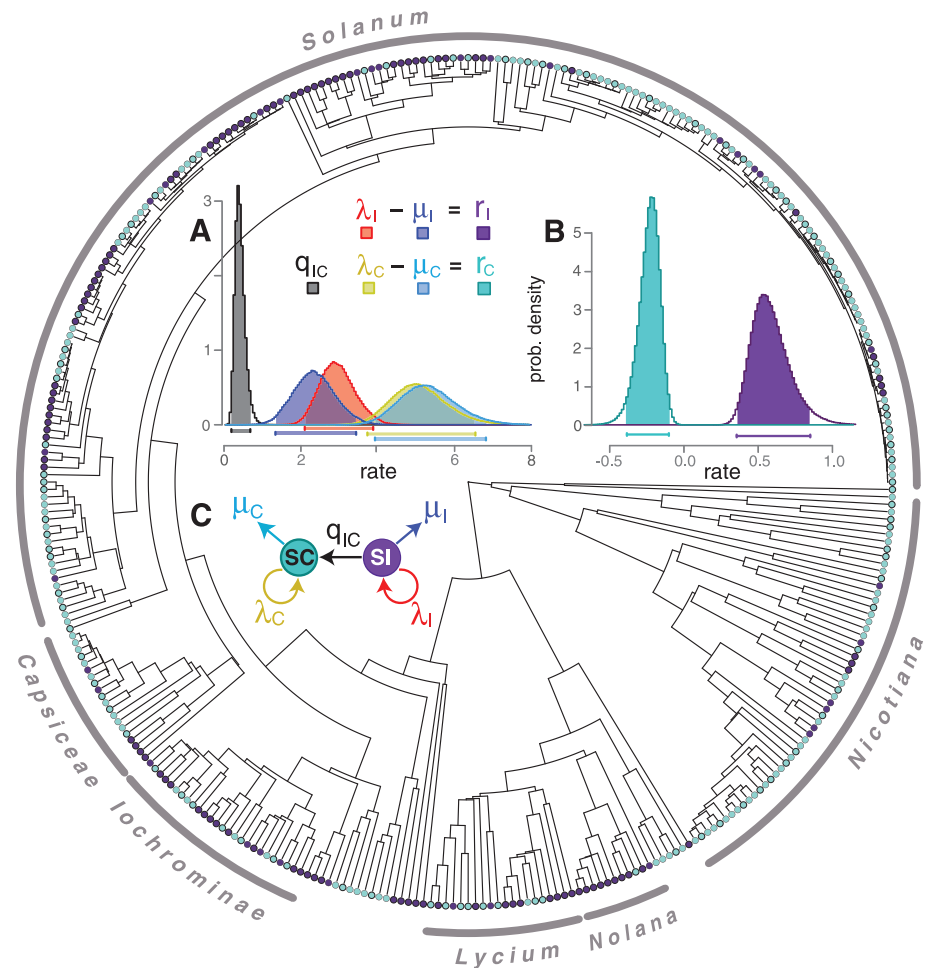


Fig. 1. Maximum likelihood tree of phylogenetic relationships among 356 species of Solanaceae. Higher ranks are indicated around the perimeter of the tree. Purple and turquoise tip colors denote SI and SC extant species, respectively. The root age is 36 million years. Inset panels display posterior probability distributions and 95% credibility intervals of reconstructed rates of character evolution (the time unit is millions of years). (A) BiSSE estimates of transition, speciation, and extinction parameters ($q_{IC} \ll \mu_1 < \lambda_1 \ll \lambda_C < \mu_C$). (B) Net diversification rate—the difference between speciation and extinction rates—associated with each state. (C) Schematic summary of estimated rate parameters. For methods, species names, character states, and further results, see (19).

and SC states, respectively), extinction (μ_I and μ_C), and loss of SI (q_{IC}) were estimated with Markov chain Monte Carlo analyses under the binary-state speciation and extinction (BiSSE) model (19, 22), which explicitly incorporates differing speciation and extinction rates associated with the two states of a binary character. Because SI has apparently not been regained in this clade (13), we fixed the rate of transitions from SC to SI, q_{CI} , to be zero in our main analyses [although we also considered bidirectional transitions (19)].

We find a higher rate of speciation for SC than SI lineages, but this apparent advantage is erased by an SC extinction rate that exceeds that of SI lineages and even the SC speciation rate [$\mu_I < \lambda_I < \lambda_C < \mu_C$, with posterior probability 0.99 (Fig. 1A)]. SI lineages consequently show a substantially higher rate of net diversification than do SC lineages [$r_I > r_C$, with posterior probability 1.0 (Fig. 1B)]. Furthermore, this diversification difference is large enough to counter the irreversible loss of SI [$r_I > r_C + q_{IC}$, with posterior probability 1.0 (Fig. 2A)]. The equilibrium frequency of SI is therefore positive [$p_{SI} > 0.25$, with posterior probability 0.99 (Fig. 2B)], demonstrating that the macroevolutionary advantage of SI is enough for it to persist indefinitely, despite its loss through transitions to SC. These results are robust to phylogenetic uncertainty and against the assumption that SI is not regained (19).

The higher rate of diversification associated with SI means that selection is acting among species, which can produce trends that are not predictable from studies of selection among individuals within species. Natural selection can act on many levels of evolutionary hierarchy (including genes, individuals, populations, and species), with the potential for cascading effects of selection from any level to those above and below (18, 23–26). Species, as units, may vary in heritable traits associated with different rates of multiplication. A higher form of selection may thus be manifested as a trait-dependent net diversification rate. There is wide acceptance of the existence of higher-order selection (18, 25, 26) but relatively sparse data on its importance (25, 26).

Our results point to a strong role for species selection in determining the evolutionary dynamics and distribution of mating systems among species. Both theoretical and empirical studies on the shape of outcrossing rate distributions have overlooked the possibility that species selection may produce an arbitrary proportion of selfing, mixed-mating, and outcrossing species (8, 27).

It remains unknown why strict avoidance of self-fertilization yields such strong long-term evolutionary advantages. Outcrossing plants, which generally maintain high effective population sizes and recombination rates, may have increased polymorphism, lower linkage disequilibrium, more efficient response to purifying selection, more extensive geographic distribution of genetic diversity, and lower rates of extinction (28–31). Various mechanisms other than SI, such as the physical and temporal separation of sexes within hermaphrodite flowers, or single-sex individuals, can effect outcrossing in the absence of SI (3). Moreover, although SI may be a relatively efficient mechanism for obligate outcrossing (32), delayed selfing ability and plastic sex expression afforded by other breeding systems may additionally provide reproductive assurance (7).

At short time scales, the expression of inbreeding depression can oppose the spread of mutations that break down SI, but many ecological factors favor their rapid and frequent fixation (7). This is problematic because natural selection cannot maintain features slated for future use (18). We find that SC is associated with a macroevolutionary disadvantage from high extinction rates, despite its high speciation rates relative to SI. Both theoretical and empirical evidence link inbreeding with an increased probability of extinction (27, 29, 31). Self-fertilization and increased inbreeding are also closely tied to higher among-population differentiation (30), which may in turn lead to escalating speciation rates.

We conclude that species selection opposes the short-term advantages provided by the ability of flowering plants to self-fertilize, and that volatile dynamics underlie the scattered phylogenetic distribution of SC plant species. A strong

relationship between traits, including SI, and speciation and extinction may result in the uneven patterns of diversification rates observed among clades of flowering plants (2, 4), especially when coupled with time heterogeneity and historical contingency. These results provide evidence that the evolutionary origin and maintenance of SI may play an important role in shaping the immense extant diversity of flowering plants (18, 33).

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Materials and Methods

SOM Text

Figs. S1 and S2

Tables S1 to S4

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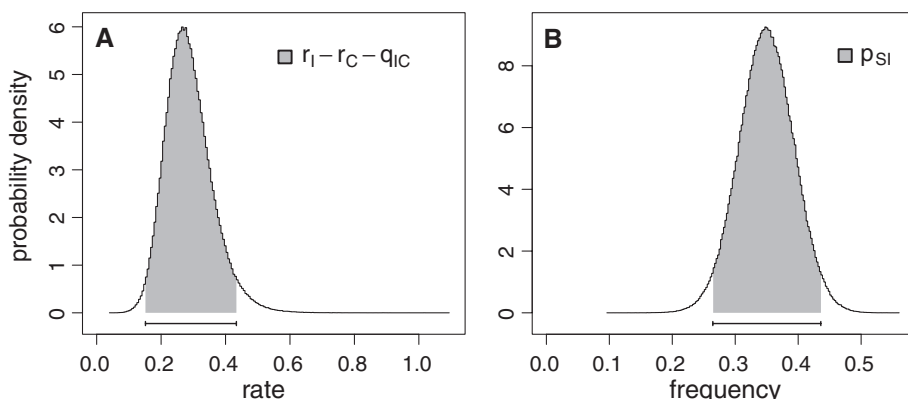


Fig. 2. Further analyses of the posterior rate distributions in Fig. 1. (A) The diversification rate of SI is sufficiently higher than that of SC for it to persist deterministically (shown by positive values here) despite frequent and irreversible loss. (B) The equilibrium frequency of SI is consequently always positive.

Impeding *Xist* Expression from the Active X Chromosome Improves Mouse Somatic Cell Nuclear Transfer

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Cloning mammals by means of somatic cell nuclear transfer (SCNT) is highly inefficient because of erroneous reprogramming of the donor genome. Reprogramming errors appear to arise randomly, but the nature of nonrandom, SCNT-specific errors remains elusive. We found that *Xist*, a noncoding RNA that inactivates one of the two X chromosomes in females, was ectopically expressed from the active X (Xa) chromosome in cloned mouse embryos of both sexes. Deletion of *Xist* on Xa showed normal global gene expression and resulted in about an eight- to ninefold increase in cloning efficiency. We also identified an *Xist*-independent mechanism that specifically down-regulated a subset of X-linked genes through somatic-type repressive histone blocks. Thus, we have identified nonrandom reprogramming errors in mouse cloning that can be altered to improve the efficiency of SCNT methods.

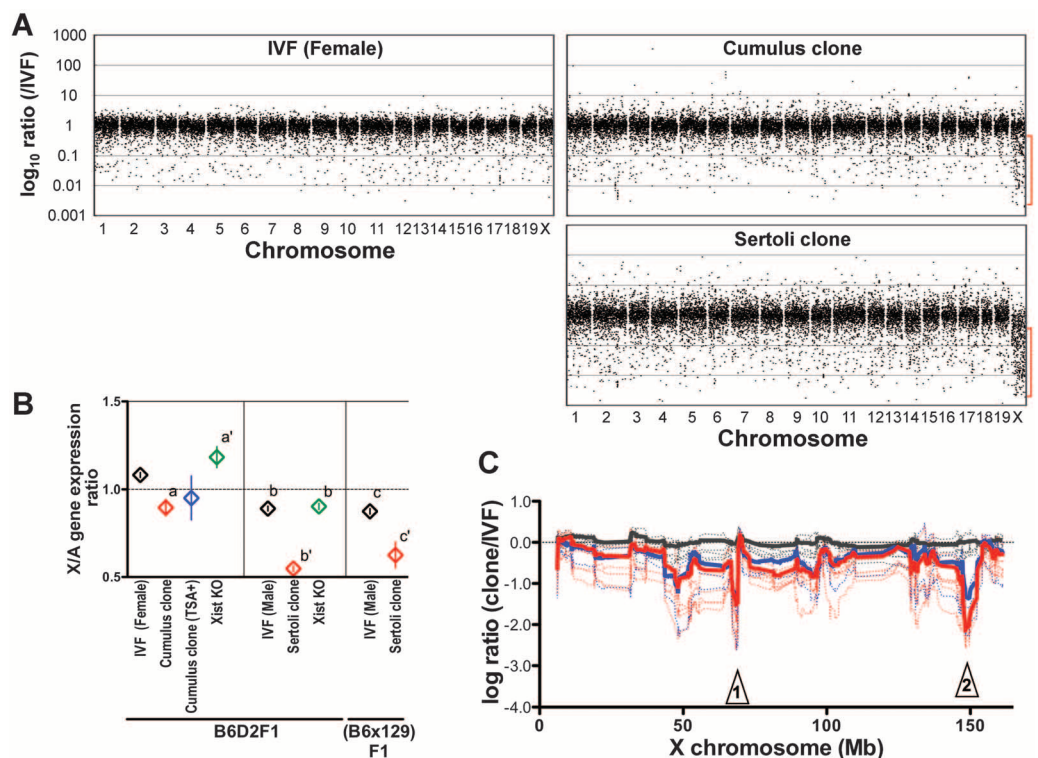
Cloned animals have been generated from embryonic cells (blastomeres) or somatic cells by nuclear transfer. The latter type of cloning, somatic cell nuclear transfer (SCNT), has more practical applications and has been applied successfully to more than 20 animal species (1). However, despite extensive efforts to improve the technique, the efficiency in terms of normal birth remains low. For example, screening for tissue-specific stem cells that might provide a more efficient donor cell has shown limited success (2, 3). The observation of many SCNT-specific pheno-

types in cloned animals, such as placental abnormalities (4) and immunodeficiency (5), led us to hypothesize that SCNT might be associated with some definable, nonrandom epigenetic errors. By combining genetics, functional genomics, and cloning technologies, we now identify nonrandom reprogramming errors in cloned embryos that provide promising clues for improving SCNT cloning.

To define the gene expression patterns specific for SCNT, we generated mouse embryos from cumulus cells and immature Sertoli cells under

standardized SCNT conditions (6). Single cloned blastocysts were analyzed for their global gene expression patterns by comparing them with genotype-matched controls produced by means of in vitro fertilization (IVF) at the same time (7). When the relative expression levels of filtered genes in cloned embryos taken from a 44,000 oligo DNA microarray were plotted on the 20 chromosomes, genes on the X chromosome were specifically down-regulated (Fig. 1A). This phenomenon was sex- and genotype-independent because the average X:autosome (X:A) expression ratio in the three types of cloned embryos (cumulus and Sertoli clones with different genotypes) was consistently lower than in the corresponding control embryos (Fig. 1B). Detailed observations on the entire X chromosome revealed that although there seemed to be some gene-specific variations, the X-linked genes were largely down-regulated in most regions (Fig. 1C). We then performed a statistical analysis using Student's *t* test to identify the number of affected genes in cloned embryos. In each clone group, 2560 to 5540 out of

Fig. 1. Large-scale down-regulation of X-linked genes in SCNT embryos. **(A)** A representative pattern of relative gene expression levels of a B6D2F1 IVF embryo, a cumulus cell cloned embryo, and a Sertoli cloned embryo, plotted on the genomic positions from chromosomes 1 to X (except for Y). The red bar indicates down-regulated X-linked genes in a cloned embryo. **(B)** The ratio of the expression levels of X-linked genes to autosomal genes. Wild-type cloned embryos, including those treated with TSA, showed lower X:A ratios as compared with the corresponding IVF controls. The data are represented as the mean $\pm$ SEM. ^a, ^a*p* < 0.01, ^b, ^b*p* < 0.0001, ^c, ^c*p* < 0.05 (one-way analysis of variance and Student's *t* test). **(C)** Relative gene expression levels of (red) cumulus cell cloned embryos, (blue) cumulus cell cloned embryos treated with TSA (*n* = 3 embryos), and (gray) IVF embryos plotted on the positions of the X chromosome. Dotted lines represent a single embryo, and solid lines indicate their mean values. Arrowheads 1 and 2 indicate the position of the *Xlr* and *Magea* clusters, respectively (Fig. 3B).



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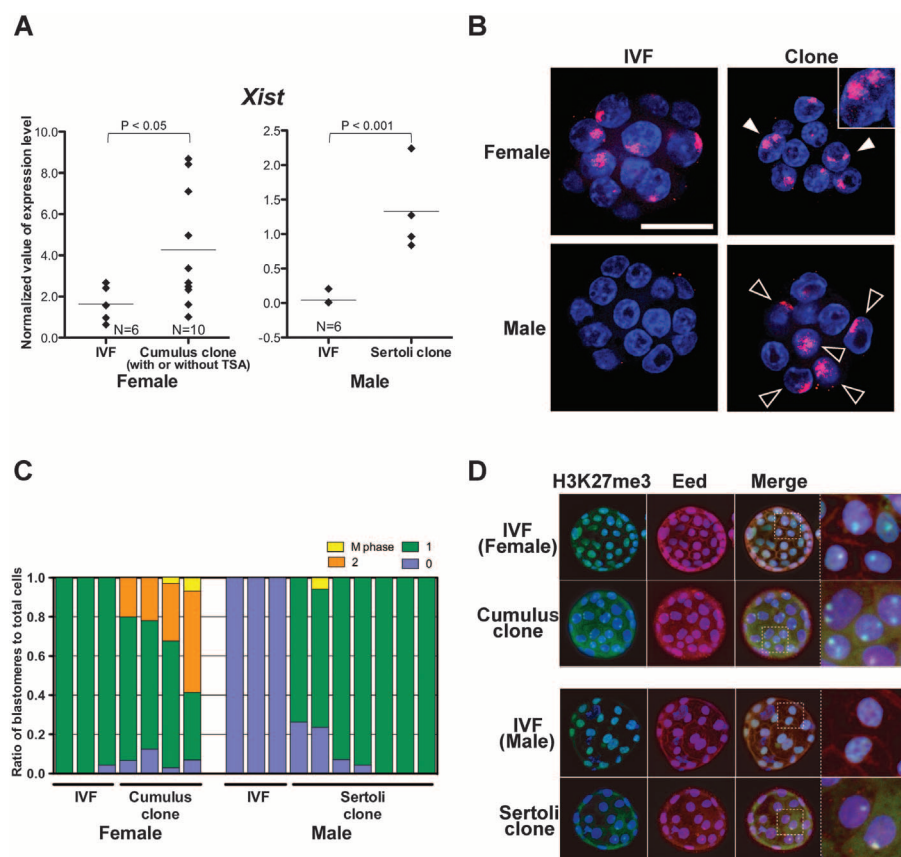
39,448 gene probes were expressed differentially as compared with that of the genotype-matched IVF controls (fig. S1A). However, the affected genes common to all the clone groups represented only 129 genes (145 probes), with 90 being up-regulated and 39 down-regulated (fig. S1B). Thus, SCNT caused dysregulation of a large subset of genes, but most followed a pattern specific to each donor cell type. Twenty-one out of 39 (54%) of the commonly down-regulated genes ("CDGs") were mapped to the X chromosome ($P < 1.0 \times 10^{-72}$ versus the expected number from the X-linked gene population with Pearson's χ^2 test) (table S1 and fig. S1C), compared with a nonbiased population of genes in the up-regulated genes ($P > 0.05$) (table S2 and fig. S1C). For some CDGs, their clone-associated down-regulation was confirmed by means of quantitative real-time polymerase chain reaction (RT-PCR) experiments (fig. S2). We also analyzed embryos cloned from fibroblasts and from blastomeres of four-cell embryos and confirmed that the X-linked down-regulation largely could be attributed to SCNT cloning and not generally to nuclear transfer cloning (fig. S3 and table S1). Next, we tested whether the X-linked down-regulation of cloned embryos could be ameliorated through treatment with trichostatin A (TSA), which is a histone deacetylase inhibitor (HDACi) known to improve mouse cloning efficiency (8). However, this treatment produced no significant improvement in the X:A expression ratio

($P > 0.05$) (Fig. 1B) or in the expression levels of X-linked genes (Fig. 1C) as compared with that of untreated cloned embryos.

The chromosome-wide gene down-regulation on the X chromosome in cloned embryos was reminiscent of X chromosome inactivation (XCI). This process normally triggers inactivation of one of the two X chromosomes in female embryos so that the gene dosage is comparable with that in males. XCI is initiated by *Xist* RNA coating in cis, although it is completed and maintained by many other molecules (Fig. 2D) (9). We then examined whether *Xist* was expressed excessively in our cloned embryos, as has been reported for embryos cloned from cumulus cell nuclei (10, 11). In both female and male cloned embryos, the *Xist* expression levels were significantly higher than in control IVF embryos ($P < 0.05$), as confirmed with microarray (Fig. 2A) and quantitative RT-PCR analyses (fig. S4A). We postulate from these findings that *Xist* was expressed ectopically from the active X chromosome (Xa) in cloned embryos. We then observed the number of *Xist* domains within each blastomere nucleus at the morula or early blastocyst stage by use of RNA fluorescent in situ hybridization (FISH). As expected, about half of the IVF embryos consistently showed a single domain in each blastomere, and the remaining half showed no domain, probably representing female and male embryos, respectively (Fig. 2, B and C). In female clones, all four embryos contained blastomeres with unusual

biallelic *Xist* domains with a variable frequency from 20.0 to 51.7% (Fig. 2, B and C). In male clones, all seven embryos analyzed showed one strong *Xist* RNA domain in the majority of blastomeres (Fig. 2, B and C), whereas their donors had no *Xist* expression (12). We could exclude the possibility of involvement of tetraploidy in this excessive number of *Xist* RNA domains because there were very few blastomeres with duplicated X chromosomes in the cloned embryos (fig. S5). We further confirmed the localization of trimethylated histone H3 at lysine 27 (H3K27me3) and of Eed, which are responsible for the repressive chromatin state in the inactive X (9). In male and female cloned embryos, they colocalized exclusively in one and two domains in the nucleus, respectively, suggesting that the ectopic *Xist* expression indeed leads to XCI (Fig. 2D). The ectopic expression of *Xist* first appeared at the four-cell stage and increased up to the blastocyst stage, as revealed through quantitative RT-PCR and RNA FISH analysis by use of male cloned embryos (fig. S6). These findings support our hypothesis that *Xist* is ectopically expressed and aberrantly inactivate Xa in both male and female clones. At present, we do not know the causes of the ectopic expression of *Xist* in cloned embryos. However, because it is assumed that the major mechanisms of genomic memory for Xi (or conversely, Xa) in preimplantation embryos and somatic cells are different (9, 13, 14), re-establishment of the Xi (or Xa) memory in the

Fig. 2. *Xist* is ectopically expressed on the active X chromosome in female and male cloned embryos. **(A)** The expression levels of *Xist* in female and male embryos. The expression levels are significantly higher in cloned embryos of both sexes ($P < 0.05$, Student's *t* test). **(B)** Morula or early blastocyst stage embryos with localizations for (red) *Xist* RNA and (blue) nucleus. Ectopic expression of *Xist* is evident from the existence of two domains in females (white arrowheads) and one domain in males for *Xist* RNA (black arrowheads). **(C)** The ratio of blastomeres classified by the number of *Xist* RNA domains within single embryos (0 to 2). Each bar represents one embryo. **(D)** Immunostaining for H3K27me3 and Eed in IVF and cloned blastocysts. The signals of H3K27me3 and Eed are colocalized in single or double domains within blastomere nuclei. There are two localizations in embryos cloned from cumulus cells (females) and one in embryos cloned from Sertoli cells (male), suggesting that the Xa chromosome is inactivated aberrantly in cloned embryos of both sexes.



somatically derived genome in reconstructed embryos might have been incomplete.

Because *Xist* has a chromosome-wide repressive effect on X-linked genes in cis, next we asked to what extent its ectopic expression might be responsible for the aberrant gene expression observed in cloned embryos. To this end, SCNT was performed by using donor cells containing an *Xist*-deficient ($X^{\Delta Xist}$) X chromosome (15) for Xa and analyzed the embryos for their gene expression patterns. In both female (cumulus cell) and male (Sertoli cell) clones, the numbers of down-regulated X-linked genes in wild-type clones were considerably decreased in $X^{\Delta Xist}$ clones by 85% (80 $\rightarrow$ 12) and 85% (141 $\rightarrow$ 21) in female and male embryos, respectively (Fig. 3A). This effect is clearly noted in the upper shift of the gene expression levels plotted on the X chromosome (Fig. 3, B and C) and A:X ratios (Fig. 1B). This had a genome-wide effect, and the numbers of down-regulated autosomal genes also decreased by 85% (461 $\rightarrow$ 71) and 73% (340 $\rightarrow$ 91) in female and male embryos, respectively (Fig. 3A). These results indicate that the ectopic *Xist* expression could have adversely affected gene expression in cloned embryos in a genome-wide manner, probably through complex gene networks connecting autosomal genes and X-linked genes that direct embryonic development. However, two discrete groups of genes

remained down-regulated (Fig. 3, B and C). These were the *Magea* and *Xlr* gene clusters localized on XqF3 and XqA7.2–7.3, respectively (Fig. 1C). Twelve of the 21 X-linked CDGs were classified into one of these two clusters [table S1 and supporting online material (SOM) text].

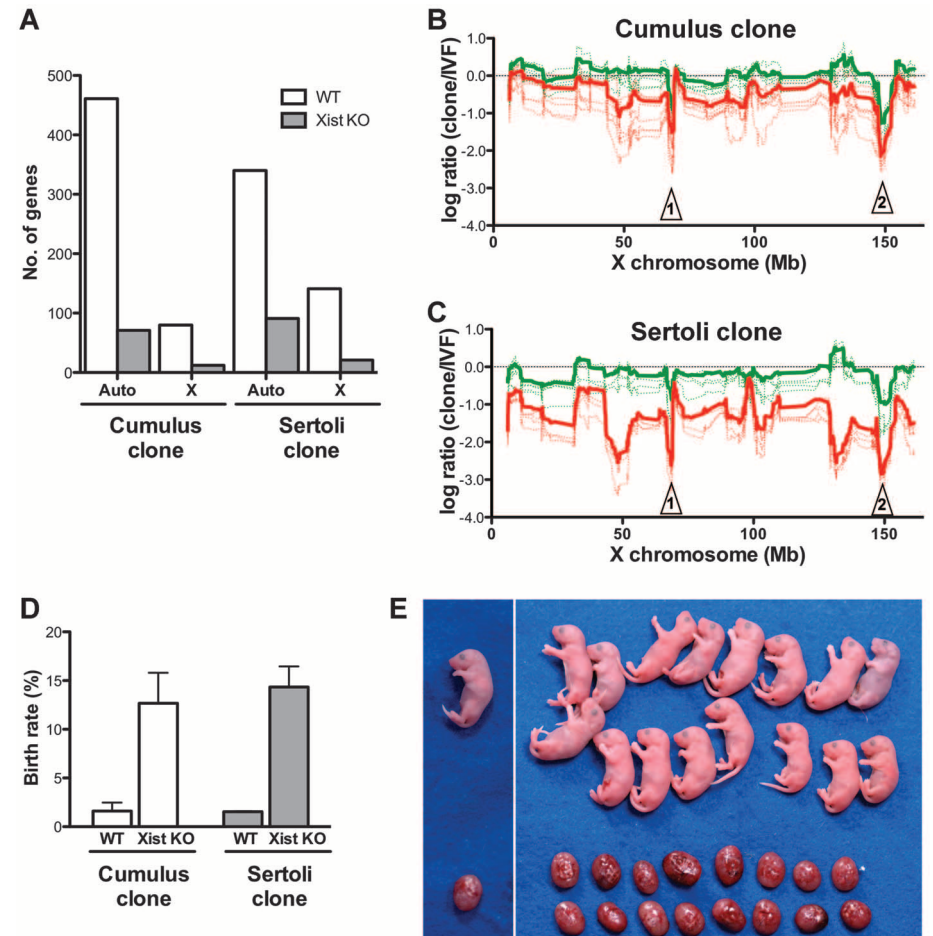
In the next series of experiments, we transferred SCNT embryos containing an *Xist*-deficient Xa into pseudopregnant recipient females. In both cumulus and Sertoli cell-derived clones, their development was greatly improved; the average birth rates reached 12.7 and 14.4% per embryos transferred (up to 19.2%), corresponding to eight- to ninefold higher levels than wild-type controls, respectively (Fig. 3, D and E, and table S3). Mouse cloning from this standard genetic strain background (B6D2F1) has not reached such high efficiencies (1, 16). Most clones grew into normal adults and showed no gross abnormalities (table S3).

In this study, we identified two types of SCNT-associated errors specifically affecting the X chromosome in mice: (i) the ectopic *Xist* expression from Xa and (ii) persistence of repressive histone modifications (H3K9me2) in the *Magea* and *Xlr* regions (SOM text). These errors were resistant to TSA treatment, indicating that they cannot be rescued by simply enhancing the accessibility of the putative ooplasmic reprogramming factors. Thus, we can broadly classify the epigenetic errors

in cloned mouse embryos into two categories: One is random and can be overcome to some extent by enhancing genomic reprogramming (such as through HDACi treatment), whereas the other is more specific and probably beyond the ability of the putative ooplasmic factors that are to reprogram the germ cell genome (17). We found that *XIST* expression was also elevated in female and male bovine SCNT embryos (fig. S4B); therefore, this could have broad implications for improving mammalian SCNT techniques. Indeed, there is a clear association between the death of bovine cloned embryos and aberrant X-linked gene expression in the placenta (18). Because the data presented in this study are still limited, it is necessary to examine whether certain genetic or epigenetic modifications for *XIST* might improve the survival of SCNT embryos using other mammalian species or mice from different strains.

A major goal of cloning research is to increase the efficiency of mammalian SCNT to a practical level (for example, >20% per embryos transferred) because of the many potential applications in biological drug manufacturing, regenerative medicine, and agriculture (19). To this end, we need to overcome the fundamental differences between somatic and germ cell genomes. We expect that cloning will become more practical by specifically targeting nonrandom epigenetic errors associated with SCNT.

Fig. 3. Deletion of *Xist* on the active X chromosome (Xa) in SCNT embryos improves their gene expression patterns and developmental ability in vivo. (A) The numbers of down-regulated genes in SCNT embryos compared with corresponding IVF embryos. With deletion of *Xist* on the Xa, they are reduced by 73 to 85% for both the X chromosomes or autosomes in both female cumulus cell and male Sertoli cell clones. (B and C) The relative expression levels of X-linked genes plotted on the X chromosome position in (B) cumulus and (C) Sertoli cell cloned embryos. The majority of down-regulated genes are increased in their expression levels, except for genes within the *Xlr* (arrowheads 1) or *Magea* (arrowheads 2) clusters, in *Xist*-knockout clones ($n = 5$ and 4 cumulus and Sertoli clones, respectively, in green), compared with (red) wild-type cloned embryos (SOM text). (D) The birth rates per embryos transferred. Eight- to ninefold increases were observed in *Xist* knockout clones. (E) Fetuses born after nuclear transfer by using Sertoli cells (left) with or (right) without the *Xist* gene on Xa. The birth rates were 1.6 and 15.4% of embryos transferred, respectively (table S2).



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20. The mice in which *Xist* had been knocked out (RBRC 01260) were provided by the RIKEN BioResource Center. This research was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology and NOVARTIS Foundation (Japan) for the Promotion of Science. The H3K9me2 antibody used for chromatin immunoprecipitation on chip was a kind gift from H. Kimura. We thank M. Tachibana, Y. Shinkai, H. Koseki, T. H. Endo, and S. L. Marjani for their invaluable suggestions. The microarray data have been deposited in the Gene Expression Omnibus and given the series accession number GSE23181.

Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S8

Tables S1 to S3

References

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Two Pairs of Neurons in the Central Brain Control *Drosophila* Innate Light Preference

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Appropriate preferences for light or dark conditions can be crucial for an animal's survival. Innate light preferences are not static in some animals, including the fruit fly *Drosophila melanogaster*, which prefers darkness in the feeding larval stage but prefers light in adulthood. To elucidate the neural circuit underlying light preference, we examined the neurons involved in larval phototactic behavior by regulating neuronal functions. Modulating activity of two pairs of isomorphic neurons in the central brain switched the larval light preference between photophobic and photophilic. These neurons were found to be immediately downstream of *pdf*-expressing lateral neurons, which are innervated by larval photoreceptors. Our results revealed a neural mechanism that could enable the adjustment of animals' response strategies to environmental stimuli according to biological needs.

Preference between light and darkness plays an important role in animal life (1–4). The fruit fly *Drosophila melanogaster* avoids light at the first to mid-third instar larval stage, but this photophobic behavior is thereafter reduced, before pupation (5–7). In addition to the circadian photoreceptor cryptochrome (CRY), the larval visual system includes two bilateral groups of 12 photoreceptors (8, 9): the Bolwig's organs (BO), which send out Bolwig's nerves (BNs) to innervate the pace-making neurons, the pigment-dispersing factor (Pdf)-expressing lateral neurons (*pdf* neurons) in larval central brain (6, 10). Blocking either BO or *pdf* neurons causes larval blindness, as measured by phototactic assay (6, 11, 12).

To investigate downstream neurons underlying larval phototactic behavior, we screened a batch of up to 800 Gal4 lines [obtained from *Drosophila* Genetic Resource Center (DGRC),

Kyoto] in a simple light-dark choice assay (6) using the Gal4/UAS system to drive ectopic expression of the tetanus toxin light chain (TeTxLC; *UAS-TNTG*), a neuron-specific toxin that prevents presynaptic release of synaptic vesicles (13). Whereas most Gal4 lines manifested photophobia and several Gal4 lines exhibited loss of light preference with ectopic TeTxLC expression at early to mid-third instar larval stage, one Gal4 line, *NP394-Gal4*, demonstrated a preference for light. This line showed positive larval phototaxis when TeTxLC expression was driven by *NP394-Gal4* [Fig. 1A, performance index (PI) = -0.35 ± 0.07 , $P < 0.001$, $n = 16$; fig. S1]. Furthermore, temporary TeTxLC expression in *NP394-Gal4*-labeled neurons was able to confer positive phototaxis at various larval stages (fig. S2). The positive larval phototaxis was reproduced by ectopic expression of a mutated form of the open rectifier potassium channel, dORKΔC (Fig. 1A, PI = -0.25 ± 0.09 , $P < 0.05$, $n = 16$), the overexpression of which hyperpolarizes neurons and subsequently inactivates neuronal function (14).

To find more phototaxis-positive Gal4 lines sharing common labeling with the *NP394-Gal4*, we rescreened all the Gal4 lines by overexpressing dORKΔC. This method was chosen because the overexpression of TeTxLC led to lethality or defects in locomotion in a large number of Gal4

lines, meaning that behavioral assays could not be conducted. Two lines, *NP423-Gal4* and *NP867-Gal4*, manifested positive larval phototaxis when the labeled neurons were inhibited by dORKΔC (Fig. 1A, PI = -0.22 ± 0.06 for *NP423-Gal4 > dORKΔC* and PI = -0.34 ± 0.07 for *NP867-Gal4 > dORKΔC*, $n = 16$ for both lines). For further confirmation at the behavioral level, we applied the temperature-sensitive form of Dynamin (*shi^{ts}*) that instantly inhibits cell endocytosis at the restrictive temperature (15). In all three Gal4 lines, ectopic expression of *shi^{ts}* at the restrictive temperature resulted in positive larval phototaxis (Fig. 1B, PI = -0.22 ± 0.05 for *NP394-Gal4 > UAS-shi^{ts}*, PI = -0.22 ± 0.05 for *NP423-Gal4 > UAS-shi^{ts}*, and PI = -0.29 ± 0.05 for *NP867-Gal4 > UAS-shi^{ts}*, $n = 16$ for all lines). By contrast, hyperactivation of *NP394-Gal4*-labeled neurons by expressing the sodium channel NaChBac (16) significantly enhanced light avoidance in late third instar larvae, which generally exhibit reduced light avoidance compared with younger larvae (figs. S3 and S4). Thus, we concluded that regulation of activity in neurons labeled by these Gal4 lines could switch larval phototaxis between negative and positive, suggesting that these neurons mediate the larval preference between light and darkness.

In parallel with behavioral screening, we used a membrane-tethered green fluorescent protein (mCD8-GFP) to visualize the expression patterns of the Gal4 lines at the larval stage. Outside the central nervous system (CNS), common labeling was found only in the salivary gland in all three Gal4 lines (table S1). In the larval CNS, the *NP394-Gal4* expression pattern was most restricted of the three Gal4 lines (Fig. 1, C to H, and fig. S5). *NP394-Gal4* labeling was most marked in two pairs of mirror-symmetrically arranged neurons in the supraesophageal ganglion from as early as the first instar and throughout larval development (fig. S6). For convenience, we refer to these cells as *NP394-neurons*. In the other two Gal4 lines, labeling of neurons with morphology and location similar to those of the *NP394-neurons* was also observed (Fig. 1, C to H). To confirm that the same *NP394-neurons* were labeled in all three Gal4 lines, we conducted combinatorial Gal4 labeling. In flies carrying combinations of two

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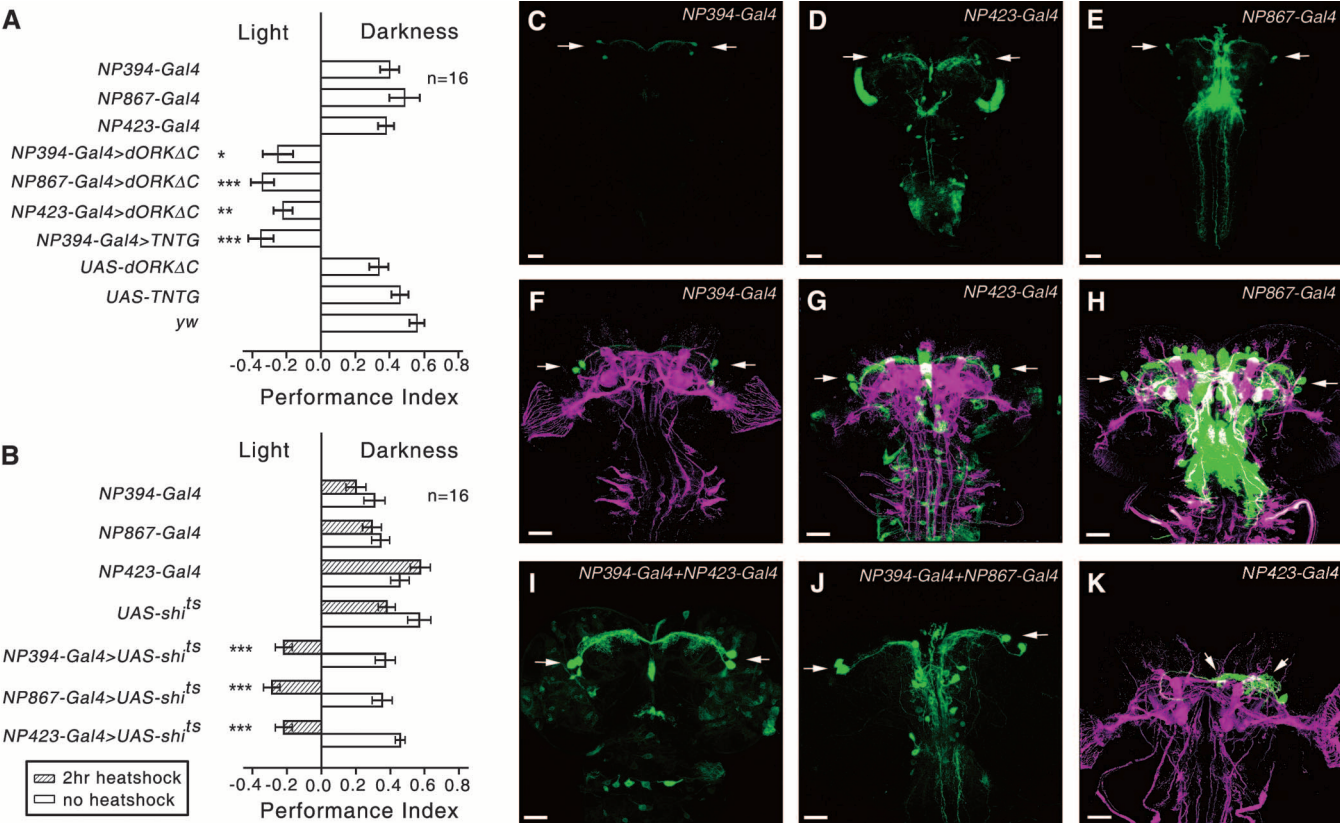


Fig. 1. Positive larval phototaxis was induced by inhibition of neurons labeled by three Gal4 lines. **(A)** Expression of TeTxLC (*UAS-TNTG*) and *dORKΔC* with the Gal4/UAS system in the three Gal4 lines led to positive larval phototaxis, whereas the Gal4 and UAS lines alone resulted in negative phototaxis. **(B)** Expression of *shi^{ts}* with the Gal4/UAS system in the three Gal4 lines led to positive larval phototaxis at restrictive temperature. Early to mid-third instar larvae were used. Data are presented as the mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001; *n* = 16 for all groups. **(C to H)**

Expression patterns of *NP394-Gal4* (**C** and **F**), *NP423-Gal4* (**D** and **G**), and *NP867-Gal4* (**E** and **H**) in third instar larval CNS. **(I and J)** Double Gal4 expression patterns of *NP394-Gal4* + *NP423-Gal4* (**I**) and *NP394-Gal4* + *NP867-Gal4* (**J**) in third instar larval CNS. **(K)** Single-neuron morphology of the *NP394-neurons* in *NP423-Gal4*. Background is FasII-labeled neural tracts (magenta). mCD8-GFP (green) was used to label the neuron. Arrows indicate the soma of the *NP394-neurons* in **(C)** to **(J)** and two arborization regions in **(K)**. Scale bars, 20 μm.

Gal4 lines such as *NP394-Gal4* and *NP423-Gal4*, or *NP394-Gal4* and *NP867-Gal4*, the number of *NP394-neuron*-like neurons visualized was the same as that in the single Gal4 line of *NP394-Gal4* (Fig. 1, **I** and **J**). Such a lack of increment in the neuron number suggests that the *NP394-neurons* were labeled by all the Gal4 lines. Thus, the *NP394-neurons* appear to be the only neurons labeled in common in the CNS in all three Gal4 lines.

We propose that the *NP394-neurons* are crucial for switching the larval light preference between darkness and light. To verify this, we introduced *elav-Gal80*, which can specifically repress Gal4 activity in neurons, into *NP394-Gal4* flies to prevent Gal4 expression in *NP394-neurons* (17). In parallel with the repression of Gal4 labeling in *NP394-neurons* by *elav-Gal80* (Fig. 2, **A** and **B**), the positive larval phototaxis in the TeTxLC-expressing *NP394-Gal4* line became negative, possibly because TeTxLC expression driven by *NP394-Gal4* was excluded from the *NP394-neurons* (Fig. 2C, PI = -0.31 ± 0.07 for *NP394-Gal4* > *UAS-TNTG*, and PI = 0.39 ± 0.09 for *NP394-Gal4* + *elav-Gal80* > *UAS-TNTG*, *n* = 16 for both lines). Thus, inhibition of the activity

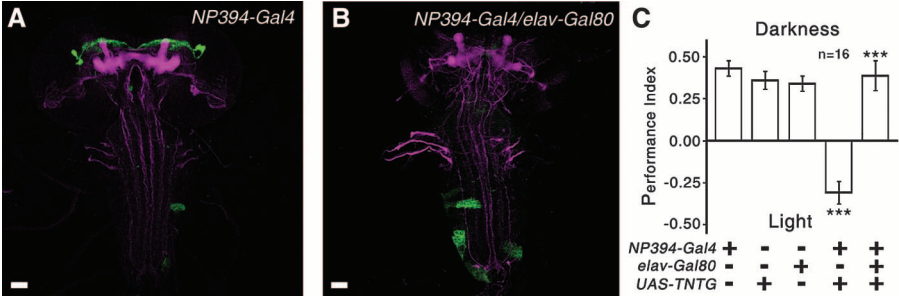


Fig. 2. Masking *NP394-neurons* from inhibition restored negative larval phototaxis. **(A and B)** Introduction of *elav-Gal80* repressed *NP394-Gal4* labeling (green) in *NP394-neurons* but left labeling in other regions intact. Anti-FasII labeling (magenta) was used as background. Scale bars, 20 μm. **(C)** Larval phototaxis changed from positive to negative when *elav-Gal80* was introduced into *NP394-Gal4* larvae expressing TeTxLC. Data are presented as the mean ± SEM. ****P* < 0.001; *n* = 16 for all groups.

of the *NP394-neurons* was able to reverse the larval light preference.

We then investigated the morphology of the *NP394-neurons*. The contralateral projections of *NP394-neurons* from both sides were largely overlapped, making single-neuron morphology difficult to identify. Hence, the FLP-out technique was applied to investigate the morphology of single *NP394-neurons* (Fig. 1K and fig. S7).

Only one type of single-neuron morphology was observed. The cell body was located in the center of each brain hemisphere. The neuron projected posteriorly for a short distance in most cases, then medially, crossing the midline along the same path as that in the corresponding contralateral *NP394-neurons* and turning posteriorly in the end. Two major arborization regions were formed: one close to the terminal region of lateral neurons

Fig. 3. Synaptic contact between *NP394-neurons* and *pdf* neurons. (A and B) Patterns of *NP394-neurons* (green) and *pdf* neurons (magenta) in larval brain. (A) Patterns in z-axis projection. (B) Patterns in a single scanning layer of 0.3 μm . Inset in (B) shows the overlap between *NP394-neurons* and *pdf* neurons in an x-z view. Arrows indicate overlapping regions. (C to G) GRASP signals visualized between *pdf* neurons and *NP394-neurons* labeled by *NP394-Gal4* (C to E), *NP423-Gal4* (F), and *NP867-Gal4* (G). GRASP signal in the framed region in (C) is shown at higher magnification in (D) and (E). Insets in (F) and (G) show only the GRASP signal. Arrows indicate the GRASP signal. Scale bars, 20 μm .

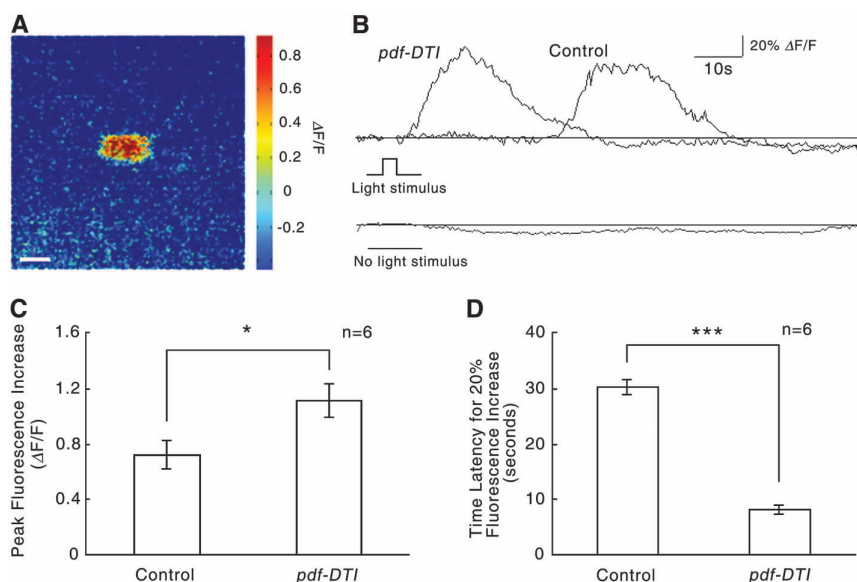
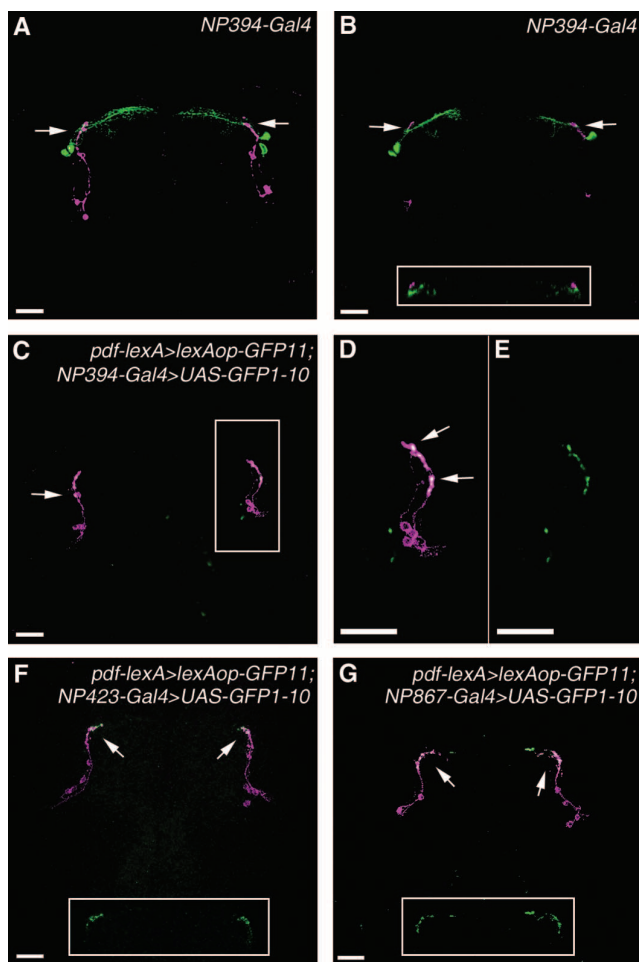


Fig. 4. Ablation of *pdf* neurons allows stronger and faster response to light stimulation in *NP394-neurons*. (A) Change in GCAMP3.0 fluorescence ($\Delta F/F$) upon light stimulation. Scale bar, 10 μm . (B) Representative response processes of *NP394-neurons* in control and *pdf-DTI* larvae upon light stimulation. (C) Average peak response in control and *pdf-DTI* larvae. (D) Average time for fluorescence change to achieve 20% (20% $\Delta F/F$) after light stimulation in control and *pdf-DTI* larvae. Data are presented as the mean $\pm$ SEM. * $P < 0.05$, *** $P < 0.001$; $n = 6$ for all groups.

labeled by an antibody against Pdf (18), and the other closer to the midline (figs. S7 and S8). The part of the tract that crossed the midline appeared to contain little arborization.

Because the *NP394-neurons* appeared to be involved in larval light preference, we speculated that they might somehow receive visual information from the larval visual sensory system. We compared the *NP394-Gal4*-labeled *NP394-neurons* with Pdf-specific antibody-labeled *pdf* neurons. The dendrites of *NP394-neurons* were close to the axonal termini of *pdf* neurons, although the interface area appeared to be limited (Fig. 3 and fig. S8). Consequently, it seems that the *NP394-neurons* are postsynaptic to *pdf* neurons. Thus, the polarity of *NP394-neurons* was examined. We expressed both the presynaptic-specific markers, such as Syt-GFP (19), and postsynaptic marker Dscam-GFP (20), in *NP394-neurons* using *NP423-Gal4* to determine the pre- and postsynaptic regions of the neurons (fig. S9). The arborization regions were not labeled by the presynaptic-specific marker Syt-GFP, but were strongly labeled by the postsynaptic marker Dscam-GFP. Thus, the arborization region of contact with the *pdf* neurons was most likely postsynaptic.

To verify the synaptic interaction between the *NP394-neurons* and the *pdf* neurons, we used the recently developed GRASP (green fluorescent protein reconstitution across synaptic partners) technique (21, 22). In this technique, the two components of GFP, GFP1-10 and GFP11, are differentially expressed in two neighboring cells, and form fluorescent reconstituted GFP across cellular gaps if the cells are close enough to each other. We expressed GFP1-10 in *NP394-neurons* and GFP11 in *pdf* neurons (23) and observed signals of reconstituted GFP in regions where *pdf* neurons may synapse with *NP394-neurons* (Fig. 3, C to G, and fig. S10). To further confirm the contact between *NP394-neurons* and *pdf* neurons, we examined the morphology of *NP394-neurons* in the absence of *pdf* neurons to test whether the presence of *pdf* neurons is required for the normal morphological development of *NP394-neurons*. We did not find any substantial morphological change of *NP394-neurons* in a *pdf-DTI* line in which *pdf* neurons in brain hemispheres were missing (fig. S11). Nevertheless, calcium imaging analysis revealed that the *NP394-neuron* response to light stimulation, as indicated by GCAMP3.0 fluorescence (24), was stronger and faster in the absence of *pdf* neurons than in their presence (Fig. 4, peak $\Delta F/F$: 0.72 ± 0.10 for control and 1.11 ± 0.12 for *pdf-DTI*; time latency for 20% $\Delta F/F$: 30.19 ± 1.44 s for control and 8.03 ± 0.73 s for *pdf-DTI*; movies S1 to S3). Thus, our results suggest that functional contact does exist between the *NP394-neurons* and *pdf* neurons. Because *pdf* neurons receive visual information necessary for phototactic behavior, we propose that the *NP394-neurons* may receive input signals from *pdf* neurons for the reversion of light preference.

As larvae with inhibited *NP394-neurons* manifested positive phototaxis whereas those with

hyperactivated *NP394-neurons* showed promoted light avoidance, we propose that *NP394-neuron* activity is positively correlated with larval light-avoidance ability. Two possible scenarios could be operating in this situation. First, the activity of *NP394-neurons* itself controls the larval phototaxis by an unknown mechanism. Second, the *NP394-neurons* activate the pathway that mediates avoidance of light whereas other unidentified neurons activate the pathway that underlies avoidance of darkness, as was shown for the mechanisms underlying odor-taxis in adult *Drosophila* (25).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6003/499/DC1
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Figs. S1 to S13
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Movies S1 to S3

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Fast Vesicle Fusion in Living Cells Requires at Least Three SNARE Complexes

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Exocytosis requires formation of SNARE [soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor] complexes between vesicle and target membranes. Recent assessments in reduced model systems have produced divergent estimates of the number of SNARE complexes needed for fusion. Here, we used a titration approach to answer this question in intact, cultured chromaffin cells. Simultaneous expression of wild-type SNAP-25 and a mutant unable to support exocytosis progressively altered fusion kinetics and fusion-pore opening, indicating that both proteins assemble into heteromeric fusion complexes. Expressing different wild-type:mutant ratios revealed a third-power relation for fast (synchronous) fusion and a near-linear relation for overall release. Thus, fast fusion typically observed in synapses and neurosecretory cells requires at least three functional SNARE complexes, whereas slower release might occur with fewer complexes. Heterogeneity in SNARE-complex number may explain heterogeneity in vesicular release probability.

The SNARE [soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor] complex formed between two fusing membranes is at the heart of the molecular machinery that mediates exocytosis (1). It is a coiled bundle of four parallel α helices provided by

three SNARE proteins: SNAP-25 (synaptosome-associated protein of 25 kD), synaptobrevin-2, and syntaxin-1 (2). SNARE-complex formation proceeds from the N- to the C-terminal end in a discontinuous process that involves partially assembled intermediates. Assembly of the most C-terminal three to four interaction layers coincides with membrane merger (3). Though it has been unclear whether assembly of one SNARE complex generates sufficient energy to initiate vesicle fusion (4–7), it was recently reported that liposomes can fuse with the help of a single SNARE complex, albeit with low speed (8). Other studies have concluded that 5 to 11 SNARE complexes might be involved in faster modes of fusion (9–13).

To study the dependence of fast vesicle fusion on higher-order SNARE complexes in intact cells,

we used an exceptionally inhibitory SNAP-25 mutation in a titration assay that allowed us to relate exocytosis to the relative expression levels of mutant and wild-type (WT) protein in a given cell. Our mutant harbored two alanine substitutions [Met⁷¹ → Ala⁷¹ (M71A) and Ile⁹² → Ala⁹² (I192A)] in the interaction layer +5, facing the inside of the complex (2). If incorporated in the SNAP-25A isoform, this mutation completely fails to reconstitute exocytosis in *Snap-25^{-/-}* adrenal chromaffin cells (14). Here, we introduced the mutation into SNAP-25B, because this isoform supports two to three times more fast-phase secretion (15). SNAP-25B WT (SN25B) or mutant protein (denoted SN25BL5**) were N-terminally fused to enhanced green fluorescent protein (EGFP) or mCherry (mCh), allowing for the quantitative analysis of expression levels and protein localization.

Using the Semliki Forest virus (SFV) expression system, we characterized mCh-tagged SN25BL5** and mCh-SN25B expressed separately in SNAP-25-deficient chromaffin cells (16). Both proteins were localized to the plasma membrane and expressed to similar levels (Fig. 1, E and F). Secretion was assayed by membrane capacitance measurements and amperometry after flash photorelease of caged calcium. Expression of mCh-SN25BL5** suppressed secretion (total capacitance change: 12 ± 3 fF after 5 s; $n = 28$ cells) (Fig. 1, A to E) compared with mCh-SN25B-infected cells (510 ± 54 fF; $n = 36$; $P < 0.0001$) and even SNAP-25-deficient cells (39 ± 5 fF; $n = 35$; $P < 0.0001$; Student's *t* test). This made SN25BL5** an attractive inhibitor for a titration experiment. Several lines of evidence indicate that inhibition probably arises from interference with a very late step of exocytosis associated with the C-terminal assembly of the SNARE complex: (i) SN25L5** forms stable SNARE complexes

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in vitro, exhibiting assembly kinetics indistinguishable from WT protein (14). However, mutant SNARE complexes exhibit a strongly destabilized C-terminal end (14). (ii) SNAP-25 is required for vesicle docking (17) before participating in downstream steps of exocytosis. Expression of SN25BL5** completely restored docking in *Snap-25*^{-/-} cells (Fig. 1, G to I, and fig. S1), excluding an involvement of layer +5 in these early steps of exocytosis. (iii) The phenotypes caused by single point mutations in layer +5 of SN25B (M71A or I192A) indicate that assembly of this layer is important for fusion triggering. Expression of

either mutant in *Snap-25*^{-/-} cells slowed down fusion kinetics (fig. S2, A and B), similar to other mutations compromising the C-terminal end of the SNARE complex (14, 18). In contrast, unchanged sustained-release rates (fig. S2Ab and S2Bb) and normal recovery between stimulations (fig. S2Ae and S2Be) suggest normal vesicle priming.

To obtain cells with varying ratios of mCh-SN25BL5** and EGFP-SN25B, we generated viruses harboring bicistronic expression units containing different “internal ribosome entry site” (IRES)-sequences (fig. S3) (16). After calibra-

tion with an EGFP-mCh fusion protein (fig. S3B), relative expression levels of the two fusion proteins were assessed by quantifying mCh and EGFP fluorescence. All viruses displayed substantial variations of expression ratios in individual cells, which were exploited to cover a wider range of expression ratios. The two fusion proteins colocalized closely (fig. S4), both on the plasma membrane and on certain intracellular structures (19).

In control recordings, EGFP-SN25B-expressing cells exhibited an average total capacitance increase of 483 ± 25 fF ($n = 125$). Progressively increasing fractional mCh-SN25BL5** expression

Fig. 1. A SNAP-25B mutant causes complete arrest of neurotransmitter release. (A to C) Averaged data for calcium uncaging experiments in *Snap-25*-deficient chromaffin cells (ko, knockout) (gray), knockout cells expressing mCh-SN25B wild type (ctrl) (black), or mCh-SN25B M71A, I192A (SN25BL5**; L5**) (red). (A) Intracellular calcium concentrations (mean $\pm$ SEM) after ultraviolet flash applied at 0.5 s. $[Ca^{2+}]_i$, intracellular concentration of Ca^{2+} . (B) Induced membrane capacitance change (ΔC_m). (C) Amperometric current (I_{amp}). (Inset) Cumulative amperometric charge (Q_{amp}). (D) Total capacitance change (mean $\pm$ SEM) reached after 5 s was significantly ($***P < 0.0001$, Student's t test) decreased by expression of SN25BL5**. (E) Plot of total capacitance change versus cellular fluorescence intensity. a.u., arbitrary units. (F) Confocal microphotographs of chromaffin cells expressing mCh-SN25B or mCh-SN25BL5**. Scale bar, 5 μ m. (G) Sample electron micrographs of *Snap-25* knockout cell and knockout cell expressing WT SN25B or SN25BL5**. Scale bars, 200 nm. (H) Number of docked vesicles and (I) total number of vesicles (mean $\pm$ SEM) in *Snap-25* knockout cells and after rescue with SN25BL5** and WT protein ($***P < 0.001$).

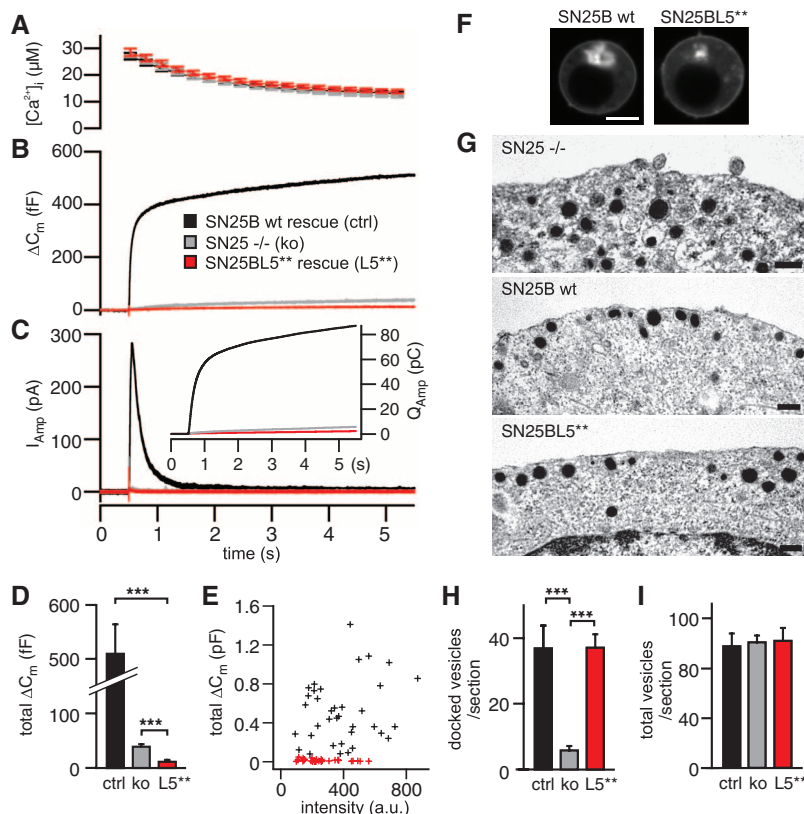
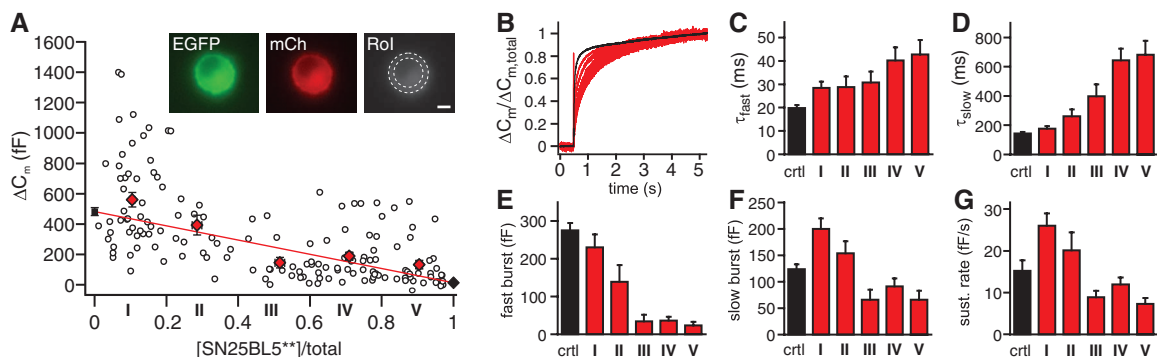


Fig. 2. Coexpression of SN25BL5** with WT SNAP-25 is accompanied by a pronounced slow-down of release. (A) Plot of the overall capacitance change (5 s after flash) versus the fraction of mutant SNAP-25 expressed. Open circles represent single recordings; red diamonds denote mean data for five (I to V) binned groups; black symbols represent the capacitance change in cells expressing only EGFP-SN25B (0%) or mCh-SN25BL5** (100%). The red line indicates a strict linear relation between a fraction of SN25BL5** and inhibition, assuming no cooperativity. Error bars represent SEM. (Inset) Microphotographs of a chromaffin cell expressing both SNAP-25 variants. Fluorescence intensity was measured in a region of interest (RoI) enclosing the plasma membrane. Scale bar, 5 μ m. (B to D) Kinetic analysis of capacitance changes. (B) Capacitance traces were

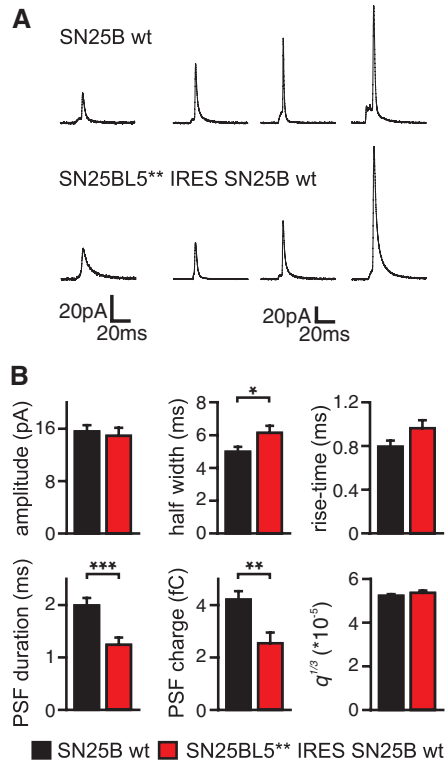


averaged in each binned group (I to V, red; EGFP-SN25B, black) and normalized to their amplitude at 5 s. (C) Fast and (D) slow bursts show gradually increasing time constants (τ_{fast} and τ_{slow} , respectively) during titration with SN25BL5**. (E) The amplitude of the fast burst steadily decreases during the titration, whereas (F) slow-burst amplitude and (G) the rate of sustained release pass through a transient maximum to eventually diminish. All values are given as mean $\pm$ SEM.

had a mild inhibitory effect on overall exocytosis (Fig. 2A). The inhibition profile was well described by a linear dependency on the fraction of mCh-SN25BL5**. Kinetic analysis revealed a progressive slowdown of release kinetics (Fig. 2B), owing to a gradual increase of fast- and slow-burst time constants (Fig. 2, C and D) combined with decreased amplitude of the fast component (Fig. 2E). For cells expressing low levels of mCh-SN25BL5** (0 to 20%, group I), the decrease in

fast-release amplitude was partly compensated for by an increase in slow-burst amplitude (Fig. 2F) and in the rate of sustained secretion (Fig. 2G). Overall, vesicles fuse at lower rates in the presence of SN25BL5**, implying that the vesicular release probability is decreased. Because exocytosis cannot be mediated by fusion complexes solely containing SN25BL5**, this finding indicates the formation of mixed fusion complexes containing both EGFP-SN25B and mCh-SN25BL5**.

Fig. 3. Altered amperometric spikes in the presence of SN25BL5** indicate the existence of heteromeric fusion complexes. (A) Examples showing amperometric spikes in control cells (*Snap-25*^{+/+} expressing EGFP-SN25B) and cells coexpressing WT and mutant SNAP-25B (mCh-SN25BL5**::IRES-EGFP-SN25B). (B) Quantitative analysis. The means of cell medians for each parameter ± SEM are shown (*q*, total charge). The half width was slightly prolonged in the presence of SN25BL5** (*P* < 0.05). The pre-spike foot duration and charge were reduced, indicating altered fusion-pore opening (***P* < 0.01 and ****P* < 0.001; Student's *t* test).



Formation of mixed fusion complexes was also supported by experiments examining single-vesicle fusion events (Fig. 3). In these experiments, *Snap-25*^{+/+} cells were infected with a SFV expressing ~80% mCh-SN25BL5** and ~20% EGFP-SN25B (fig. S3D). In the presence of mCh-SN25BL5**, amperometric spikes exhibited a slightly delayed overall waveform and a decreased duration of pre-spike feet (PSF). Because mCh-SN25BL5** cannot support exocytosis on its own, these data indicate single-vesicle fusion using mixed complexes. A similar reduction in PSF duration has been described upon mutating the C-terminal layers of synaptobrevin-2 (18).

The above findings suggested that multiple SNARE complexes might surround the nascent fusion pore (12). Thus, we asked how many SNARE complexes are needed for fast vesicle fusion. Assuming that the incorporation of one copy of SN25BL5** is sufficient to decrease the vesicular release rate, vesicle fusion with normal fast kinetics should be mediated by complexes containing exclusively WT SN25B. The number of such vesicles depends on the fractional availability of WT SN25B raised to a power of *n*, where *n* is the number of SN25B molecules in the fusion complex (20). To determine the amount of fusion with unperturbed kinetics, we repeated the kinetic analysis, but this time we fixed the fast time constant τ_{fast} to the control value (EGFP-SN25B, $\tau_{\text{fast}} = 19.8$ ms), such that the amplitude reports unperturbed secretion. The number of fast-fusing vesicles diminished much faster than overall secretion with an increasing proportion of mCh-SN25BL5** (Fig. 4A, compare with Fig. 2A). Fitting of a simple binomial model (21) to the entire curve identified *n* = 2.8. Alternatively, we fitted a straight line to the left part of the curve, following logarithmic transform of the burst am-

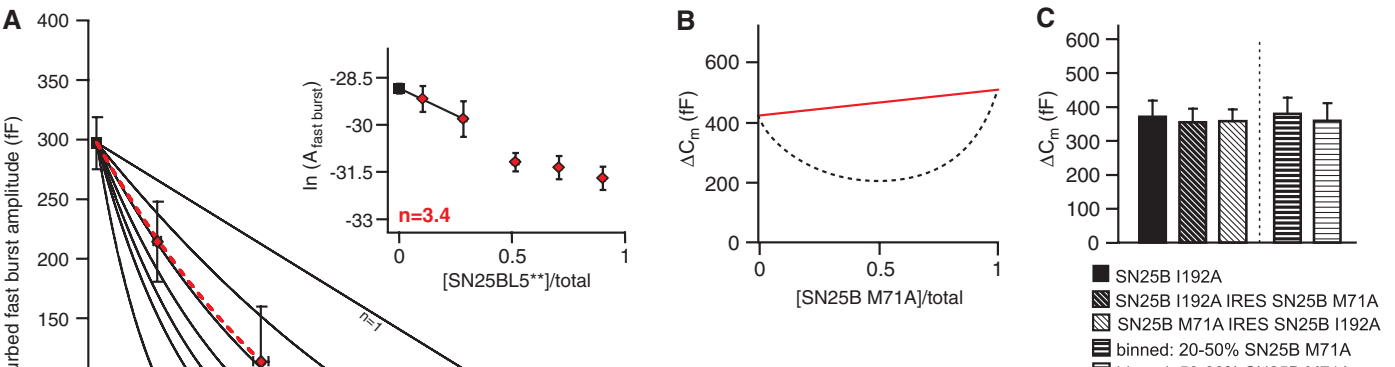


Fig. 4. Fast-fusing vesicles display higher-order dependence on the availability of SNAP-25. (A) Plot of unperturbed fast-burst amplitude versus SN25BL5** fraction (mean ± SEM). Fit of a binomial model (20) yielded *n* = 2.8 or 3.4 after the logarithmic transform, respectively (inset), interpreted as the number of SNARE complexes driving fast vesicles. The black square denotes fast-burst amplitude (*A*_{fast burst}), as derived for WT SN25B-expressing control cells. (B) If SNAP-25 cross-links SNARE complexes, coexpression of both single mutations should cause maximal inhibition at a 1:1 ratio of the two mutants (dashed line); otherwise, secretion should follow a linear interpolation line (red solid line). (C) Secretion was not inhibited in cells coexpressing SN25BM71A and SN25BI192A compared with reference recordings (SN25B I19A). Error bars indicate mean ± SEM. Examining data in the 20-to-50% or 50-to-80% expression bins also did not reveal any inhibition.

plitude (21), which yielded $n = 3.4$ (inset, Fig. 4A). Our model assumes that the affinity of SN25BL5** to the rest of the fusion apparatus is unchanged, which appears likely from previous data (14). Three is a lower estimate of the number of SNARE complexes in a fusion complex driving fast fusion, because incorporation of more than one mutant might be required to detectably change fusion kinetics.

SNAP-25 harbors two SNARE domains and could possibly contribute these to different SNARE complexes, thereby cross-linking them (21, 22). This would separate the two single mutations and mask a dominant-negative effect of SN25BL5** in the presence of WT protein (fig. S5A), which could provide an alternative explanation for the shallow dependence of overall secretion on SN25BL5** fraction (Fig. 2A). We tested such “domain-swapping” by coexpression of the two single-layer +5 mutants (M71A and I192A). At similar expression levels, the two single alanines should recreate the catastrophic double-layer +5 mutation in half of the complexes (fig. S5B), which should result in a 50% drop in secretion (Fig. 4B, according to Fig. 2A). Using two bicistronic SFVs that express both mutants at the proportions [mCh-SN25M71A]/total of $15 \pm 1\%$ or $65 \pm 3\%$, we observed no inhibitory effect on secretion (Fig. 4C). In addition, examining data in 20-to-50% or 50-to-80% expression bins did not identify any block of release (Fig. 4C). Thus, domain-swapping cannot explain the mild inhibition by SN25BL5**, nor can it represent a prominent event during exocytosis, consistent with the finding that separated SNAP-25 SNARE domains support in vitro vesicle fusion (23) and secretion (24).

Using a titration approach in intact cells, we report here that the apparent cooperativity for fast-phase secretion is higher (~ 3) than that for overall exocytosis (~ 1). We conclude that SNARE complexes form higher-order functional units, and at least three SNARE complexes are required for the fast phase of exocytosis (fig. S5, C and D). Our findings agree with data from infusion of synaptobrevin fragments into PC12 cells (11). The linear titration profile of overall secretion might be explained if stoichiometry of fusion complexes is not fixed. Vesicles resident at the plasma membrane have time to form several SNARE complexes in the absence of stimulation, achieving faster speeds of fusion when triggered by calcium. However, vesicles arriving during conditions of sustained high calcium concentrations might fuse using fewer [or possibly only a single (8)] SNARE complexes. The dramatic shift in release rate upon coexpression of SN25BL5** suggests that the number of functional (that is, completely zippering) SNARE complexes is a determinant of fusion probability. Indeed, variable fusion stoichiometry might underlie heterogeneity in vesicular release probabilities between synapses (25) or release phases (26) and could represent an important regulated parameter in neurotransmitter-releasing cells.

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Mechanisms of Proton Conduction and Gating in Influenza M2 Proton Channels from Solid-State NMR

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The M2 protein of influenza viruses forms an acid-activated tetrameric proton channel. We used solid-state nuclear magnetic resonance spectroscopy to determine the structure and functional dynamics of the pH-sensing and proton-selective histidine-37 in M2 bound to a cholesterol-containing virus-envelope-mimetic membrane so as to better understand the proton conduction mechanism. In the high-pH closed state, the four histidines form an edge-face π -stacked structure, preventing the formation of a hydrogen-bonded water chain to conduct protons. In the low-pH conducting state, the imidazoliums hydrogen-bond extensively with water and undergo microsecond ring reorientations with an energy barrier greater than 59 kilojoules per mole. This barrier is consistent with the temperature dependence of proton conductivity, suggesting that histidine-37 dynamically shuttles protons into the virion. We propose a proton conduction mechanism in which ring-flip–assisted imidazole deprotonation is the rate-limiting step.

Proton transport in synthetic materials is mediated either solely by hydrogen-bonded (H-bonded) water, as in hydrated ionic polymers (1), or solely by titratable heterocycles, such as imidazoles tethered to the backbone of

anhydrous polymers (2). In comparison, the conduction mechanism of biological proton channels in cell membranes is more complex because both water and titratable protein sidechains are usually present (3). The influenza M2 protein forms a tetrameric proton channel that is important for the virus life cycle (4). Activated below pH 6, the M2 channel conducts 10 to 10,000 protons per second (5, 6). The pH-sensing and proton-selective residue is a single histidine, His37, in the trans-

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membrane (TM) domain (7). ^{15}N chemical shifts of His 37 in 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)/dimyristoyl phosphatidylglycerol (DMPG) bilayers indicated that the four histidines titrate with pK_a s of 8.2, 8.2, 6.3, and <5.0 (where K_a is the acid dissociation constant); thus, the third protonation event is responsible for channel activation (8). However, the precise role of His 37 in proton conduction is still debated. Two models have been proposed. In the “shutter” model, the pore at His 37 is enlarged through electrostatic repulsion among the imidazoliums, permitting a continuous H-bonded water chain over which protons hop by means of the Grotthuss mechanism (9, 10). The rate-limiting step is proton-hopping across three or four charged imidazoliums, with a calculated energy barrier of 29 to 42 kJ/mol (9). In the “shuttle” model, His 37 actively participates in proton relay through protonation and deprotonation. Tautomerization or ring flips reestablish the original conformation required for the next proton relay (11). The rate-limiting step in this model is the His 37 conformational change.

Although high-resolution structures of the M2 TM domain (M2TM) in detergents at high and low pH have been reported (12, 13), the His 37 sidechain conformations differed in these structures, and sidechain dynamics and water interactions were not probed. Further, detergent molecules can perturb the packing of weakly bound membrane protein complexes; thus, the structures may not accurately reflect the chemistry of the imidazoles in the lipid membrane.

To elucidate the proton conduction mechanism of M2, we used solid-state nuclear magnetic resonance (NMR) to determine the structure and dynamics of His 37 in M2TM reconstituted into a cholesterol-rich virus-envelope-mimetic lipid membrane (14, 15). Extensive data yielded the His 37 protonation state, tautomerization, rotameric conformation, sidechain dynamics, and hydrogen bonding from pH 8.5 to 4.5. Here, we focus on pH 8.5 for the closed channel and pH 4.5 for the conducting channel. M2TM exhibits

acid-activated and amantadine-sensitive proton currents similar to the intact protein (16) and fully assembles into four-helix bundles (17) in the viral membrane with immobilized backbones (14), which allowed His 37 sidechain motion to be elucidated.

Histidine ^{15}N and ^{13}C chemical shifts are exquisitely sensitive to the protonation state and tautomeric structure of imidazoles. Deprotonation increases the ^{15}N chemical shift by ~80 parts per million (ppm) (18), and C γ /C $\delta 2$ chemical shifts also systematically depend on the imidazole structure (19). Two-dimensional (2D) ^{13}C - ^{13}C and ^{15}N - ^{13}C correlation spectra of His 37 -labeled M2TM revealed only neutral imidazoles at pH 8.5. The Ne2-protonated τ -tautomer and N $\delta 1$ -protonated π -tautomer exist at a ~3:1 ratio (Fig. 1), with slow or no exchange at ambient temperature (fig. S1). Inter-tautomer C $\delta 2(\tau)$ -C $\gamma(\pi)$ and C $\delta 2(\tau)$ -C $\delta 2(\pi)$ cross peaks (Fig. 1A) indicate that both tautomers exist in each channel. The ~25% fraction of the π -tautomer is much higher than in small imidazole-containing compounds (18), suggesting stabilization of the protonated N $\delta 1(\pi)$ through hydrogen bonding (20).

At pH 4.5, both N $\delta 1$ and Ne2 exhibit protonated chemical shifts (170 to 180 ppm); no unprotonated signal was observed at 250 ppm (fig. S1), indicating that the neutral species is below the detection limit (<5%). The charged imidazoliums showed much larger linewidths than the neutral species (table S1), indicating broader conformational distribution of the protein at low pH.

We probed interhelical packing of the His 37 tetrad through χ_1 - and χ_2 -dependent backbone-sidechain distances. The C α -N $\delta 1$ distance constrains the χ_2 torsion angle, whereas the C $\delta 2$ -N α distance constrains both χ_1 and χ_2 angles. At both pH, the C α -N $\delta 1$ distance was 3.9 Å (Fig. 2 and fig. S2), which ruled out the +60° and -60° χ_2 rotamers and was consistent only with the 180° rotamer. Similar experiments yielded a C $\delta 2$ -N α distance of 4.4 to 4.9 Å (fig. S3), which ruled out the $\chi_1 = -60^\circ$ rotamer. Thus, the high-pH τ -tautomer adopts the *tt* rotamer, which is consistent with interhelical His 37 -Trp 41 (21) and Trp 41 -Trp 41 distances (17). At pH 4.5, the C α -N $\delta 1$ (3.9 Å), C α -Ne2 (4.4 Å), and C $\delta 2$ -N α (>4.5 Å) distances similarly indicated the *tt* conformation (Fig. 2B and fig. S3).

Fig. 2. His 37 rotameric conformation from C α -N $\delta 1$ distances. (A) pH 8.5 data, with representative rotational-echo double-resonance control (S_0), dephased (S), and difference (ΔS) spectra. The 3.9 Å distance indicates $\chi_2 = 180^\circ$. (B) pH 4.5 data, showing a similar distance and χ_2 angle. (C) Top and side views of the His 37 tetrad in the *tt* rotamer in the high-pH structure [Protein Data Bank (PDB) number 2KQT] (22). (D) Top view of the His 37 tetrad in the *tt* rotamer in the low-pH structure (PDB number 3C9J) (13).

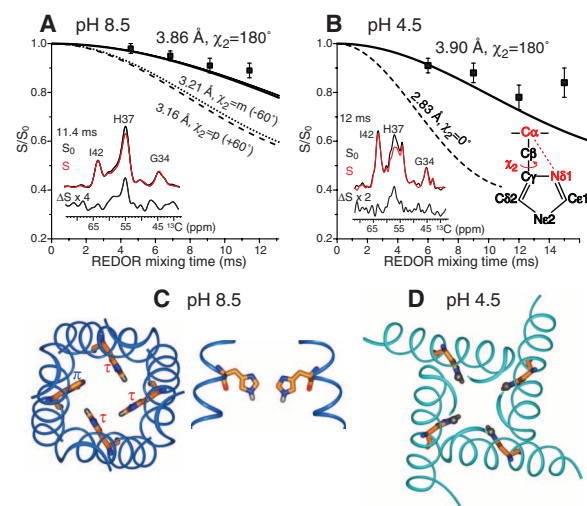
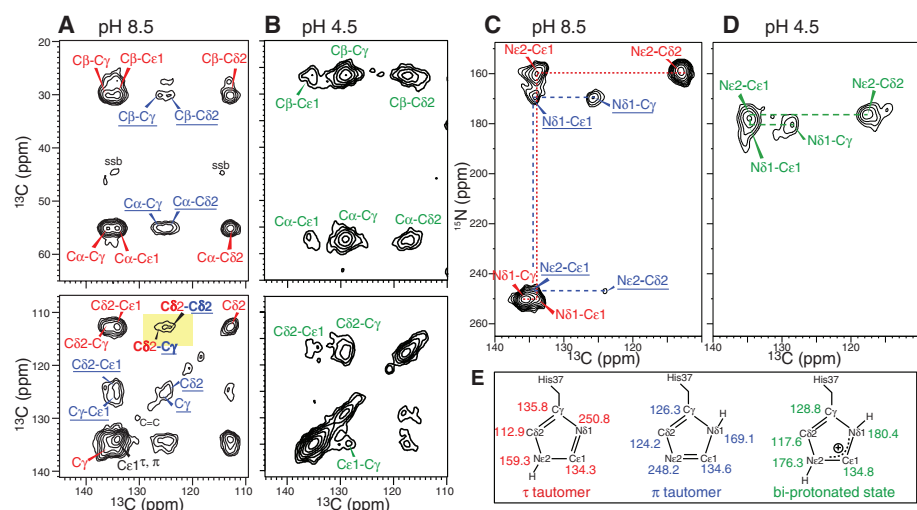


Fig. 1. ^{15}N and ^{13}C chemical shifts of His 37 -labeled M2TM in viral membranes reveal pH-dependent imidazole protonation state and tautomeric structures. (A and B) 2D ^{13}C - ^{13}C correlation spectra, (A) pH 8.5 and (B) pH 4.5. The τ - and π -tautomer peaks are assigned in red and blue, and the charged His 37 peaks are in green. (C and D) 2D ^{15}N - ^{13}C correlation spectra, (C) pH 8.5 and (D) pH 4.5. (E) Summary of the imidazole chemical shifts.



Placing the His³⁷ rotamer into the different backbone structures at low pH and high pH revealed substantially different packing of the imidazole tetrad (Fig. 2, C and D). In the closed channel (22), the major τ -tautomers pack in an edge-face fashion in which each C ϵ 1-H ϵ 1 bond points to the center of the neighboring ring. The packing is tight, with a nearest-neighbor C ϵ 1-N ϵ 2 distance of $\sim$ 4.9 Å, which is consistent with inter-tautomer cross peaks and suggests aromatic CH- π interaction (23). The high density of π -electrons should repel water oxygens and orient them in opposite directions across the tetrad, thus disabling proton conduc-

tion. The tetrad dimension is still possible for metal-ion coordination (24), which may explain Cu²⁺ inhibition of M2 (25). The minor π -tautomer can readily maintain the fourfold symmetry by adopting tt or $t0$ rotamer. In comparison, in the low-pH structure (13) the imidazoliums show no edge-face stacking and leave a much wider pore.

The considerable packing difference suggests that His³⁷ sidechains may be immobilized at high pH but dynamic at low pH. To test this hypothesis, we measured one-bond C γ -N δ 1 and C δ 2-H δ 2 dipolar couplings at physiological temperature. The viral membrane immobilized the

protein backbone, giving N α -H and C α -H α order parameters of 1.0 (figs. S4 and S5) (14), thus isolating potential sidechain motion. Fast motions scale the couplings by an order parameter (S) that reflects the motional amplitude. At pH 8.5, we obtained rigid-limit C γ -N δ 1 and C ϵ 1-N δ 1 couplings (1.15 kHz and 1.39 Å) and a rigid-limit C δ 2-H δ 2 coupling (23.9 kHz and 1.08 Å) (Fig. 3, A and B), confirming immobilization of the neutral imidazoles by edge-face stacking. However, at pH 4.5 the C γ -N δ 1 and C δ 2-H δ 2 couplings are scaled by a factor of 0.85 and 0.80, respectively (Fig. 3C), indicating sidechain motion. The availability of two-order parameters constrained the geometry of the imidazolium motion. The most likely motional axis is the C β -C γ bond (26). Uniaxial rotation is ruled out because it predicts a very small $S_{C\gamma-N\delta1}$ of 0.06 because of the 57° angle of the C γ -N δ 1 bond to the C β -C γ axis. The well-known 180° ring flip motion is also ruled out because it has little effect on the C δ 2-H δ 2 coupling ($S_{C\delta2-H\delta2} = 0.94$) (table S2). The near-invariance of the C δ 2-H δ 2 coupling to 180° ring flips also rules out a scenario in which some imidazoliums undergo ring flips whereas others remain static (fig. S6). Instead, analysis of the S dependence on χ_2 angles shows that only two-site jumps with a χ_2 change of 45° satisfies both the C γ -N δ 1 and C δ 2-H δ 2 order parameters (fig. S7). Given the average χ_2 of 180° at low temperature, the most likely instantaneous χ_2 angles are about 160° and -155° (Fig. 3D).

The restricted nature of the imidazolium ring reorientation may result from the symmet-

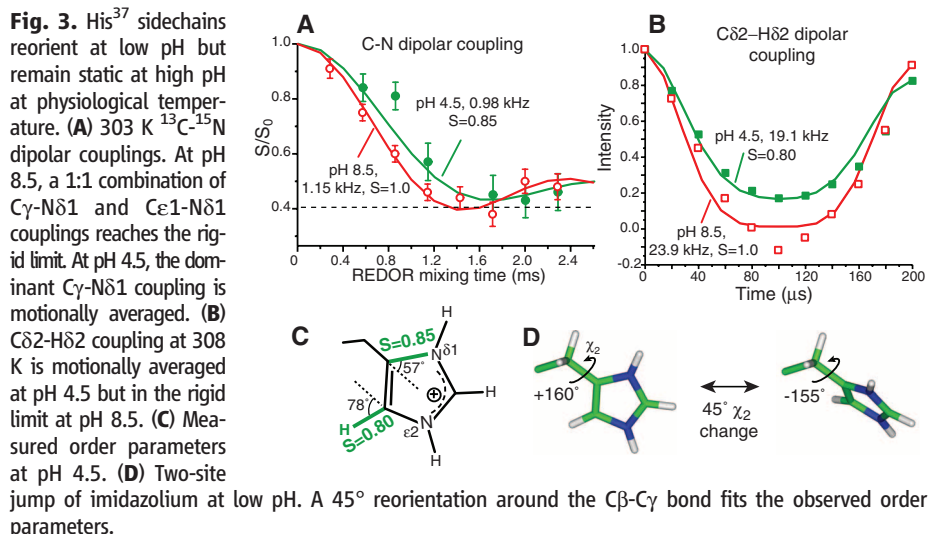
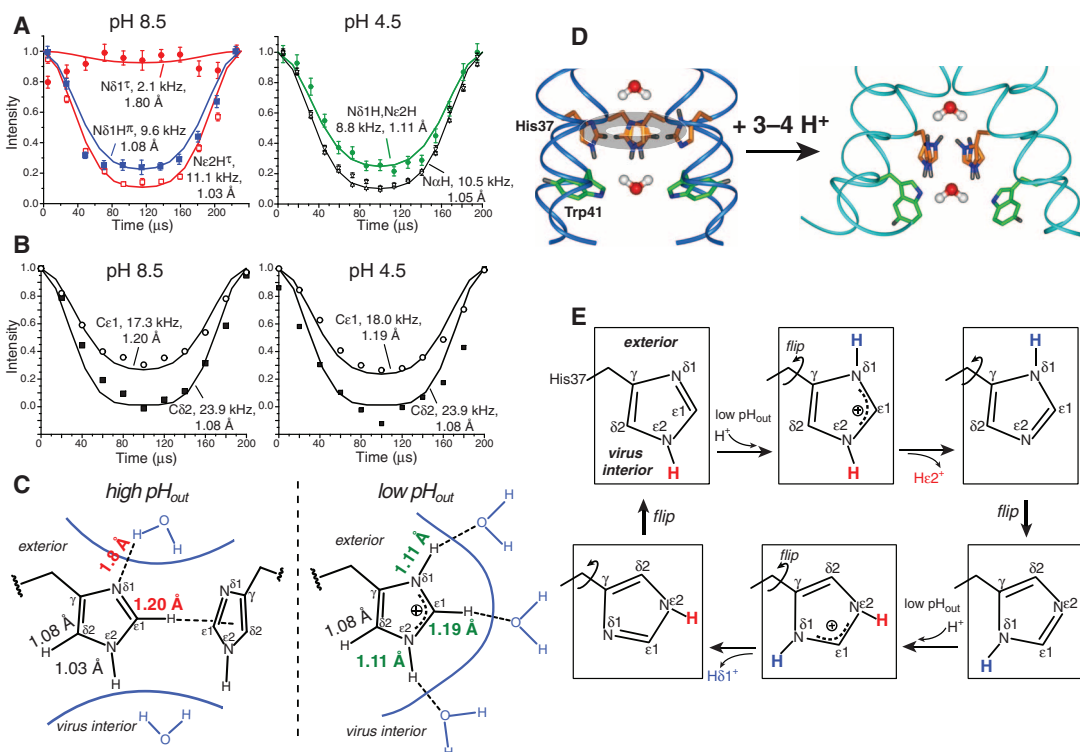


Fig. 4. Charged His³⁷ H-bonds with water and undergoes ring flips to relay protons. **(A)** N-H dipolar couplings at 243 K. At pH 8.5, N ϵ 2(τ) is not H-bonded, whereas N δ 1(τ) is. The unprotonated N δ 1(τ) shows a weak H-bond. At pH 4.5, both nitrogens show weak couplings and bond stretching. **(B)** C-H dipolar couplings of C δ 2 and C ϵ 1 at 243 K. The C ϵ 1-H ϵ 1 bond is stretched, whereas C δ 2-H δ 2 is not. **(C)** Imidazole bond lengths and H-bond networks at high and low pH. **(D)** His³⁷ structure and dynamics and proposed water orientations across the tetrad. Trp41 may interact with His³⁷ at high pH_{out}. **(E)** Proposed imidazole structural changes in a cycle in which multiple ring reorientations mediate the transfer of two protons.



ric low pH across the bilayer in the NMR samples because the imidazoles may not need to substantially reorient to be deprotonated and reprotonated. When a proton concentration gradient exists, such as in the virus membrane, full ring flips may occur. The motion must be much faster than 10^4 s^{-1} to average the C82-H82 coupling. Temperature-dependent C82-H82 couplings from 308 to 243 K indicated that the imidazolium was frozen by 263 K but fully mobile at 293 K (fig. S8). Using a lower-limit of 50 kHz for the 293 K rate and an upper-limit of 3 kHz for the 263 K rate, we obtained an energy barrier of $>59 \text{ kJ/mol}$, which is consistent with the 50 to 120 kJ/mol reported for imidazole motions in synthetic proton conductors (27). M2 proton conductivities differ by 14-fold between 18° and 37°C at pH 5.7 (5), indicating an energy barrier of 104 kJ/mol. Thus, the barrier of imidazolium motion is consistent with the functional data, whereas the barrier for water-mediated proton hopping (29 to 42 kJ/mol) is not (9), suggesting that His³⁷ ring reorientation is directly involved in proton transport, as in the “shuttle” model. Imidazole motion was also observed at the physiological pH of 6.0 and 5.2, at which the channel first opened and both charged and neutral histidines were present (fig. S8) (7, 8). Thus, His³⁷ motion appears to be an intrinsic property of the spacious conducting channel, although its precise amplitudes and rates may vary with pH.

Water is still necessary for delivering protons to the imidazoles before they can be relayed to the virus interior. Thus, water-His³⁷ hydrogen bonding is implied in the shuttle model. We probed H-bond formation by measuring imidazole N-H and C-H dipolar couplings at 243 K, at which the sidechain was frozen. H-bond formation stretches the N-H and C-H bonds from their covalent lengths (1.03 Å and 1.10 Å), thus weakening dipolar couplings (28). At pH 8.5, the protonated N81(π) showed a significantly stretched N-H bond of 1.08 Å, indicating hydrogen bonding and explaining the π -tautomer stabilization. Even the unprotonated N81(τ) showed a sizeable coupling of 2.1 kHz, suggesting a nearest-proton distance of 1.8 Å and a weak N81...H-O H-bond. In contrast, the protonated Ne2(τ) exhibited an unstretched bond length of 1.03 Å (11.1 kHz) (Fig. 4A), despite the presence of a small amount of water in the H37-W41 region on the basis of Ne2(τ)-water cross peaks in 2D ^{15}N - ^1H correlation spectra (fig. S9). At pH 4.5, the combined N81/Ne2 peak showed a reduced N-H coupling of 8.8 kHz, indicating a stretched bond of 1.11 Å. Given the spaciousness of the low-pH pore, the H-bond acceptors cannot be another imidazolium. Trp⁴¹-His³⁷ aromatic interaction may partly contribute to Ne2-H bond stretching (10, 29), but we propose the most likely cause for N-H bond elongation at low pH is H-bond with frozen water, which is more abundant in the low-pH chan-

nel than the high-pH channel, as shown with spin diffusion NMR (30) and molecular dynamics simulations (9, 31).

Similar C-H coupling measurements revealed that the C82-H82 bond was unstretched (1.08 Å) at either pH, whereas the Cε1-He1 bond was stretched to 1.20 Å at pH 8.5 and 1.19 Å at pH 4.5 (Fig. 4B). The latter may be attributed to CH- π interactions at high pH and H-bond with water at low pH. The imidazole Cε1-He1 bond is known to be prone to elongation because of its acidic nature (32), although the large magnitude of stretching observed here is surprising and requires further investigation.

These bond lengths reveal an extensive H-bond network that covers three sides of the imidazolium at low pH (Fig. 4C), creating a continuous H-bonded chain. Similar to the histidine in the catalytic triad of serine proteases, Cε1 hydrogen bonding may facilitate Ne2 deprotonation by evenly distributing the positive charge and increasing Ne2 electronegativity (26, 33). At high pH, the H-bond network is incomplete, excluding Ne2, which we attribute to the opposing water orientation and possible His³⁷-Trp⁴¹ interactions (Fig. 4D).

Taken together, these data suggest the following mechanism for proton gating and conduction by M2 (Fig. 4, D and E). At high pH_{out}, the neutral imidazoles form tightly packed electron-rich CH- π stacks, preventing the formation of a H-bonded water chain. The outward-facing N81(π) H-bonds with water, whereas the inward-facing Ne2 does not. Lowering pH_{out} protonates N81, resulting in several imidazoliums per channel, which repel each other and cause backbone conformational changes that widen the pore (34, 35). More water permeates this region (30), establishing a H-bonded chain that includes His³⁷. The larger pore frees the imidazoliums to undergo microsecond ring reorientations. We propose a proton conduction mechanism in which imidazolium deprotonation is facilitated by Cε1-He1 hydrogen bonding and continuous ring flips achieve the dual purpose of properly aligning the charged imidazolium with the C-terminal water molecules so as to cause proton transfer and then pointing the unprotonated nitrogen to the low-pH extracellular side to be reprotonated. Our data indicate that the highest energy barrier of this process is the imidazolium motion, which may account for the temperature dependence of M2 proton conductance, possibly in combination with an additional small barrier for proton transfer (5). This dynamically assisted proton transfer model is consistent with the observed deuterium isotope effect, whose magnitude also suggested a mixed H-bonded chain with dissimilar elements (6). Thus, the present data strongly suggest that His³⁷ is actively involved in proton conduction by M2. The structural information obtained here is largely invisible to conventional high-resolution techniques and demonstrates the ability of solid-state NMR

to elucidate functionally important membrane protein dynamics and chemistry.

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Insight into the Mechanism of the Influenza A Proton Channel from a Structure in a Lipid Bilayer

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The M2 protein from the influenza A virus, an acid-activated proton-selective channel, has been the subject of numerous conductance, structural, and computational studies. However, little is known at the atomic level about the heart of the functional mechanism for this tetrameric protein, a His³⁷-Trp⁴¹ cluster. We report the structure of the M2 conductance domain (residues 22 to 62) in a lipid bilayer, which displays the defining features of the native protein that have not been attainable from structures solubilized by detergents. We propose that the tetrameric His³⁷-Trp⁴¹ cluster guides protons through the channel by forming and breaking hydrogen bonds between adjacent pairs of histidines and through specific interactions of the histidines with the tryptophan gate. This mechanism explains the main observations on M2 proton conductance.

Proton conductance by the M2 protein (with 97 residues per monomer) in influenza A is essential for viral replication (1). An M2 mutation, Ser³¹ → Asn, in recent flu seasons and in the recent H1N1 swine flu pandemic renders the viruses resistant to antiviral drugs, amantadine and rimantadine (2). Previous structural determinations of M2 focused primarily on its transmembrane (TM) domain, residues 26 to 46 (3–8). Although the TM domain is capable of conducting protons, residues 47 to 62 following the TM domain are essential for the functional integrity of the channel. Oocyte assays showed that truncations of the post-TM sequence result in reduced conductance (9). Here, we report the structure of the “conductance” domain, consisting of residues 22 to 62, which in liposomes conducts protons at a rate comparable to that of the full-length protein in cell membranes (10, 11) and is amantadine-sensitive (fig. S1). This structure, solved in uniformly aligned 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine:1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine bilayers by solid-state nuclear magnetic resonance (NMR) at pH 7.5 and 30°C, shows striking differences from the structure of a similar construct (residues 18 to 60) solubilized in detergent micelles (12).

The structure is a tetramer (fig. S2), with each monomer comprising two helices (Fig. 1A). Residues 26 to 46 form a kinked TM helix, with the N-terminal and C-terminal halves having tilt angles of ~32° and ~22° from the bilayer normal, respec-

tively. The kink in the TM helix occurs around Gly³⁴, similar to the kinked TM-domain structure in the presence of amantadine (4). The amphipathic helix (residues 48 to 58) has a tilt angle of 105°, similar to that observed for the full-length protein (13) and resides in the lipid interfacial region (Fig. 1B). The turn between the TM and amphipathic helices is tight and rigid, as indicated by substantial anisotropic spin interactions for Leu⁴⁶ and Phe⁴⁷ (their resonances lie close to the TM and amphipathic helical resonance patterns, respectively; see fig. S3). The result is a structural base formed by the four amphipathic helices that stabilizes the tetramer.

The pore formed by the TM-helix bundle is lined by Val²⁷, Ser³¹, Gly³⁴, His³⁷, Trp⁴¹, Asp⁴⁴, and Arg⁴⁵, which include all of the polar residues of the TM sequence. The pore is sealed by the TM helices (Fig. 1B) and constricted by Val²⁷ at the N-terminal entrance and by Trp⁴¹ at the C-terminal exit (Fig. 1C). The gating role of Trp⁴¹ has long been recognized (14), and recently Val²⁷ was proposed to form a secondary gate (15). An important cavity between Val²⁷ and His³⁷ presents an amantadine-binding site (3, 7, 15). This drug-binding site is eliminated in a structure solubilized in detergent micelles (12) because of a much smaller tilt angle of the TM helices (16).

The linewidths of the NMR spectra (fig. S3) are much narrower for the conductance domain than for the TM domain (17), indicating substantially reduced conformational heterogeneity and higher stability (see also fig. S2). In the structure determined here, numerous nonpolar residues of the amphipathic helices extend the hydrophobic interactions interlinking the monomers, with their close approach facilitated by the small Gly⁵⁸ (Fig. 1D). In particular, Phe⁴⁸ interacts with Phe⁵⁵ and Leu⁵⁹ of an adjacent monomer and Phe⁵⁴ interacts with Leu⁴⁶ of another adjacent monomer.

The starting residues of the amphipathic helix, Phe⁴⁷ and Phe⁴⁸, are a sequence motif known to signal association of the helix with a lipid bilayer

(18). The burial of the hydrophobic portion of the amphipathic helix in the tight intermonomer interface is consistent with hydrogen-deuterium exchange data showing it to be the slowest-exchanging region for the full-length protein in a lipid bilayer (13). Furthermore, the Ser⁵⁰ hydroxyl here, which in the native protein is a palmitoylated Cys⁵⁰ residue, is located at an appropriate depth in the bilayer (at the level of the glycerol backbone; see Fig. 1B) for tethering the palmitic acid. A third native-like aspect of the amphipathic helix is the outward projection of the charged residues Lys⁴⁹, Arg⁵³, His⁵⁷, Lys⁶⁰, and Arg⁶¹ (Fig. 1B), which conforms to the “positive inside rule” such that M2 interacts favorably with negatively charged lipids in native membranes. The C termini of the amphipathic helices are situated to allow for the subsequent residues of the full-length protein to form the tetrameric M1 binding domain. Contrary to the lipid interfacial location determined here, the detergent-solubilized structure has the four amphipathic helices forming a bundle in the bulk aqueous solution where the amides fully exchange with deuterium (12).

On the external surface at the C terminus of the TM helix, a hydrophobic pocket, with the Asp⁴⁴ side chain at the bottom, has been described as a binding site for rimantadine (12). In the structure determined here, the large tilt of the TM helices widens the hydrophobic pocket, which is filled by the side chains of Ile⁵¹ and Phe⁵⁴ in the amphipathic helix, preventing accessibility to the Asp⁴⁴ side chain from the exterior (Fig. 1D). Consequently, the formation of a rimantadine-binding site on the protein exterior is likely an artifact of the detergent environment used for that structural characterization.

The heart of acid activation and proton conductance in M2 is the tetrameric His³⁷-Trp⁴¹ cluster, referred to here as the HxxxW quartet (14, 19, 20). The pK_a values for the His³⁷ residues in the TM domain solubilized in a lipid bilayer were determined as 8.2, 8.2, 6.3, and <5.0 (20). At pH 7.5 used here, the histidine tetrad is doubly protonated; each of these two protons is shared between the N_{δ1} of one histidine and the N_{ε2} of an adjacent histidine, giving rise to substantial downfield ¹⁵N chemical shifts and resonance broadening for the protonated sites (20) indicative of a strong hydrogen bond (21, 22). The structure of the histidine tetrad as a pair of imidazole-imidazolium dimers is shown in Fig. 2A. In each dimer, the shared proton is collinear with the N_{δ1} and N_{ε2} atoms (Fig. 2B); the two imidazole rings are within the confines of the backbones, to be nearly parallel to each other—a less energetically favorable situation than the perpendicular alignment of the rings in imidazole-imidazolium crystals and in computational studies (23–25). The two N_{δ1} and two N_{ε2} sites of the histidine tetrad not involved in the strong hydrogen bonds are protonated and project toward the C-terminal side (Fig. 2, A and B). The N_{ε2} protons interact with the indoles of the Trp⁴¹ residues (Fig. 2A), and the two N_{δ1} protons form hydrogen bonds with their

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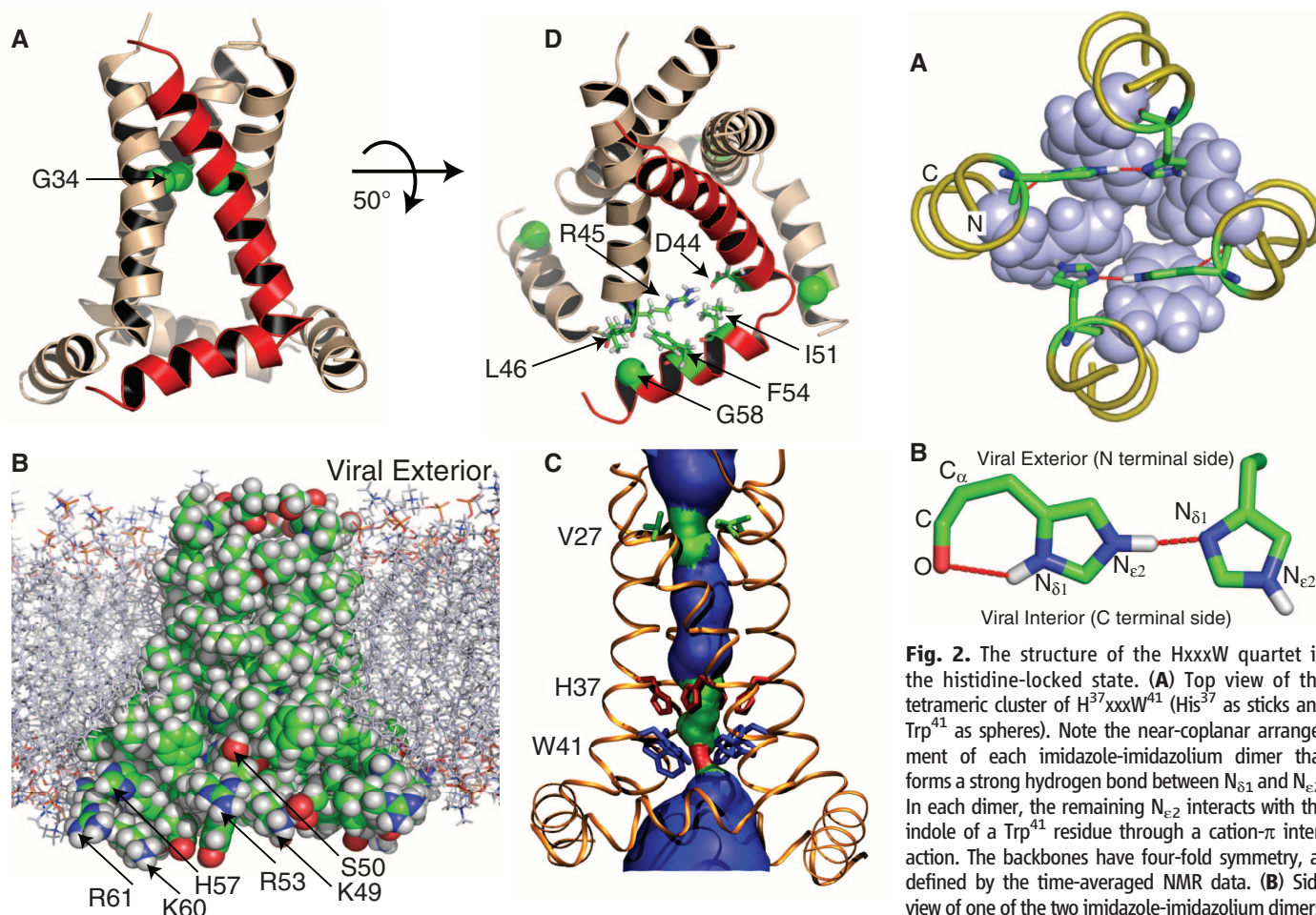


Fig. 1. The tetrameric structure of the M2 conductance domain, solved by solid-state NMR spectroscopy and restrained molecular dynamics simulations, in liquid crystalline lipid bilayers. See (32) for details and fig. S3 for the NMR spectra. **(A)** Ribbon representation of the TM and amphipathic helices. One monomer is shown in red. The TM helix is kinked around the highly conserved Gly³⁴ (shown as C_α spheres). **(B)** Space-filling representation of the protein side chains in the lipid bilayer environment used for the NMR spectroscopy, structural refinement, and functional assay. C, O, N, and H atoms are colored green, red, blue, and white, respectively. The nonpolar residues of the TM and amphipathic helices form a continuous surface; the positively charged residues of the amphipathic helix are arrayed on the outer edge of the structure in optimal position to interact with charged lipids. The Ser⁵⁰ hydroxyl is also shown to be in an optimal position (as Cys⁵⁰) to accept a palmitoyl group in native membranes. **(C)** HOLE image (33) illustrating pore constriction at Val²⁷ and Trp⁴¹. **(D)** Several key residues at the junction between the TM and amphipathic helices, including Gly⁵⁸ (shown as C_α spheres), which facilitates the close approach of adjacent monomers, and Ile⁵¹ and Phe⁵⁴, which fill a pocket previously described as a rimantadine-binding site (12).

respective histidine backbone carbonyl oxygens (Fig. 2B). Therefore, in this “histidine-locked” state of the HxxxW quartet, none of the imidazole N-H protons can be released to the C-terminal pore, resulting in a completely blocked channel. Furthermore, the only imidazole nitrogens available for additional protonation are the sites involved in the strong hydrogen bonds; acceptance and release of protons from the N-terminal pore by these sites, coupled with 90° side-chain χ_2 rotations, would allow the imidazole-imidazolium dimers to exchange partners (fig. S4). That the NMR data show a symmetric average structure suggests that such exchange occurs on a submillisecond time scale.

The structure of the HxxxW quartet at neutral pH suggests a detailed mechanism for acid activation and proton conductance (Fig. 3). Under acidic conditions in the viral exterior, a hydronium ion in the N-terminal pore attacks one of the imidazole-imidazolium dimers. In the resulting “activated” state, the triply protonated histidine tetrad is stabilized by a hydrogen bond with water at the newly exposed N_{δ1} site on the N-terminal side and an additional cation- π interaction with a Trp⁴¹ residue at the N_{ε2} site on the C-terminal side. Strong cation- π interactions between His³⁷ and Trp⁴¹ were observed by Raman spectroscopy in the TM domain under acidic conditions (26). These interactions protect the protons on the C-terminal

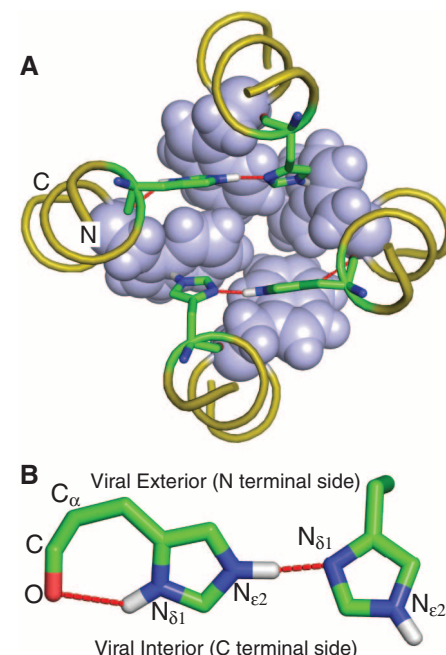


Fig. 2. The structure of the HxxxW quartet in the histidine-locked state. **(A)** Top view of the tetrameric cluster of H³⁷xxxW⁴¹ (His³⁷ as sticks and Trp⁴¹ as spheres). Note the near-coplanar arrangement of each imidazole-imidazolium dimer that forms a strong hydrogen bond between N_{δ1} and N_{ε2}. In each dimer, the remaining N_{ε2} interacts with the indole of a Trp⁴¹ residue through a cation- π interaction. The backbones have four-fold symmetry, as defined by the time-averaged NMR data. **(B)** Side view of one of the two imidazole-imidazolium dimers. Both the intraresidue N_{δ1}-H-O hydrogen bond and the interresidue N_{ε2}-H-N_{δ1} strong hydrogen bond can be seen. The near-linearity of the interresidue hydrogen bond is obtained at the expense of a strained C_α-C_β-C_γ angle (enlarged by ~10°) of the residue on the left.

side from water access. Conformational fluctuations of the helical backbones [in particular, a change in helix kink around Gly³⁴ (27)] and motion of the Trp⁴¹ side chain could lead to occasional breaking of this cation- π interaction. In the resulting “conducting” state, the N_{ε2} proton becomes exposed to water on the C-terminal side, allowing it to be released to the C-terminal pore. Upon proton release, the histidine-locked state is restored, ready for another round of proton uptake from the N-terminal pore and proton release to the C-terminal pore. In each round of proton conductance, the changes among the histidine-locked, activated, and conducting states of the HxxxW quartet can be accomplished by rotations (<45° change in χ_2 angle) of the His³⁷ and Trp⁴¹ side chains, which are much smaller than those envisioned previously (28) and even less than those needed for the imidazole-imidazolium dimers to exchange partners (fig. S4).

This proposed mechanism is consistent with many M2 proton conductance observations. The permeant proton is shuttled through the pore via

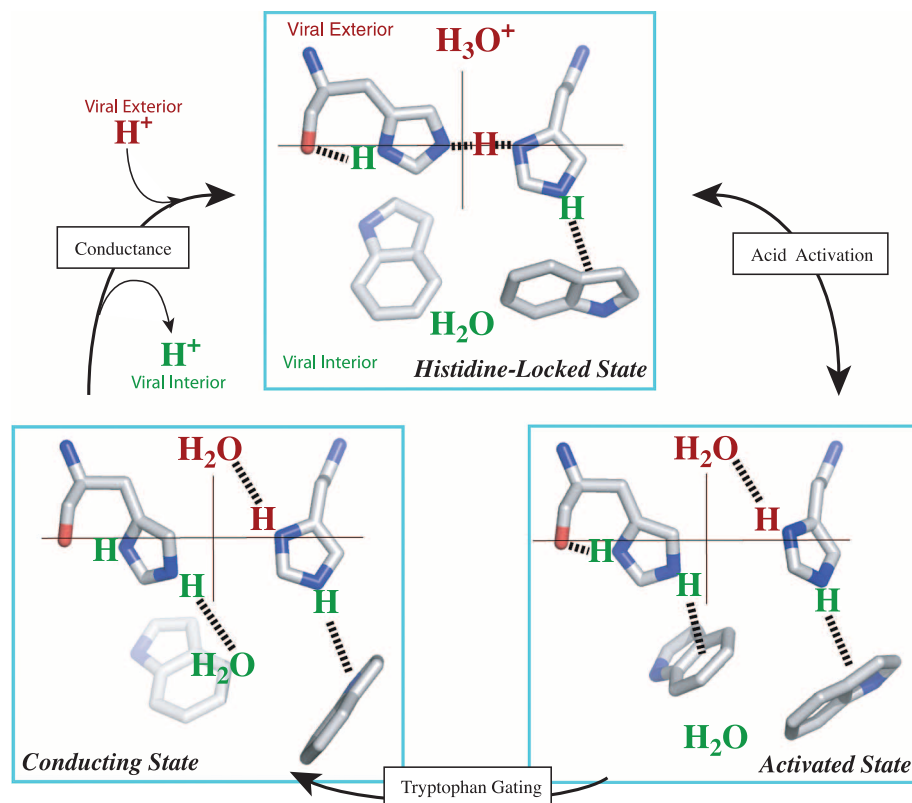


Fig. 3. Proposed mechanism of acid activation and proton conductance illustrated with half of the HxxxW quartet from a side view. The histidine-locked state (top) is shown with a hydronium ion waiting in the N-terminal pore. Acid activation is initiated with a proton transfer from the hydronium ion into the interresidue hydrogen bond between N_{δ1} and N_{ε2}. In the resulting activated state, the two imidazolium rings rotate so that the two nitrogens move toward the center of the pore; in addition, the protonated N_{δ1} forms a hydrogen bond with water in the N-terminal pore while the protonated N_{ε2} moves downward (via relaxing the C_α-C_β-C_γ angle) to form a cation-π interaction with an indole, thereby blocking water access from the C-terminal pore. The conducting state is obtained when this indole moves aside to expose the N_{ε2} proton to a water in the C-terminal pore. It was suggested previously (27) that the indole motion involves ring rotation coupled to backbone kinking. Once the N_{ε2} proton is released to C-terminal water, the HxxxW quartet returns to the histidine-locked state.

the histidine tetrad, at one point being shared between N_{δ1} and N_{ε2} of adjacent histidines. No other cations can make use of this mechanism, which explains why M2 is proton-selective. With the permeant proton obligatorily binding to and then unbinding from an internal site (i.e., the histidine tetrad), the proton flux is predicted to saturate at a moderate pH on the N-terminal side (27), which is consistent with conductance observations (29). Such a permeation model also predicts that the transition to saturation occurs at a pH close to the histidine-tetrad pK_a for binding or unbinding the permeant proton. Indeed, the transition is observed to occur around pH 6 (29), close to the third pK_a of the histidine tetrad. Without the histidines, as in the H37A mutant, the proton flux would lose pH dependence, as observed (19). Another distinguishing feature of the M2 proton channel is its low conductance, at ~100 protons per tetramer per second (fig. S1) (10, 11). Upon acid activation, the HxxxW quartet is primarily in the activated state. Only when the Trp⁴¹ gate opens occasionally to form

the conducting state is the proton able to be released to the C-terminal pore, thus explaining the low conductance.

In our mechanism, the histidine tetrad senses only acidification of the N-terminal side. This provides an explanation for an observation of Chizhnikov *et al.* (30) when they applied a positive voltage to drive protons outward in M2-transformed MEL cells. A step increase in the bathing buffer pH from 6 to 8 (with the intracellular pH held constant at pH 6) produced a brief increase in outward current, as expected for the added driving force by the pH gradient (31); however, the outward current quickly decayed to a level lower even than that before the pH increase. In our structure, when the pH in the N-terminal side is 8, the HxxxW quartet is stabilized in the histidine-locked state and the Trp⁴¹ gate prevents excess protons in the C-terminal pore from activating the histidine tetrad. Upon removal of the Trp⁴¹ gate (e.g., by a W41A mutation), a substantial outward current would be produced, as was observed (14). A related observation is that

the M2 proton channel can be blocked by Cu²⁺ (through His³⁷ coordination) applied extracellularly, but not intracellularly (14). Again, the triply protonated histidine tetrad stays predominantly in the activated state, in which the Trp⁴¹ gate blocks Cu²⁺ access to His³⁷ from the C-terminal side. However, the W41A mutation opens that access (14).

The structure of the M2 conductance domain solved in liquid crystalline bilayers has led to a proposed mechanism for acid activation and proton conductance. The mechanism takes advantage of conformational flexibility, both in the backbones and in the side chains, which arises in part from the weak interactions that stabilize membrane proteins. M2 appears to use the unique chemistry of the HxxxW quartet to shepherd protons through the channel.

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31. The brief increase can be attributed to the release of protons from the triply protonated histidine tetrad to the

N-terminal pore (as illustrated by the back arrow from the activated state to the histidine-locked state in Fig. 3) when the pH there is suddenly increased from 6 to 8. With these protons released, the histidine tetrad then becomes doubly protonated and the tryptophan gate becomes closed.

32. See supporting material on Science Online.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6003/509/DC1

Materials and Methods

Figs. S1 to S4

References

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Widespread Divergence Between Incipient *Anopheles gambiae* Species Revealed by Whole Genome Sequences

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The Afrotropical mosquito *Anopheles gambiae* sensu stricto, a major vector of malaria, is currently undergoing speciation into the M and S molecular forms. These forms have diverged in larval ecology and reproductive behavior through unknown genetic mechanisms, despite considerable levels of hybridization. Previous genome-wide scans using gene-based microarrays uncovered divergence between M and S that was largely confined to gene-poor pericentromeric regions, prompting a speciation-with-ongoing-gene-flow model that implicated only about 3% of the genome near centromeres in the speciation process. Here, based on the complete M and S genome sequences, we report widespread and heterogeneous genomic divergence inconsistent with appreciable levels of interform gene flow, suggesting a more advanced speciation process and greater challenges to identify genes critical to initiating that process.

Population-based genome sequences provide a rich foundation for “reverse ecology” (1). By analogy to reverse genetics, reverse ecology uses population genomic data to infer the genetic basis of adaptive phenotypes, even if the relevant phenotypes are not yet known. This approach can be especially powerful for gaining insight into the genetic basis of ecological speciation, a process whereby barriers to gene flow evolve between populations as by-products of strong, ecologically based, divergent selection (2). Here, we apply reverse ecology to study incipient speciation within *Anopheles gambiae*, one of the most efficient vectors of human malaria. The complex population structure of *A. gambiae*,

exemplified by the emergence of the M and S molecular forms (3), poses substantial challenges for malaria epidemiology and control, as underlying differences in behavior and physiology may affect disease transmission and compromise anti-vector measures. Genome-wide analysis of M and S can provide insight into the mechanisms promoting their divergence and open new avenues for malaria vector control.

Morphologically, M and S are indistinguishable at all life stages and can only be recognized by fixed differences in the ribosomal DNA genes (3). Geographically and microspatially, both forms co-occur across much of West and Central Africa (4), and in areas where they are sympatric, adults may be found resting in the same houses and even flying in the same mating swarms (5, 6). Assortative mating limits gene flow between forms (5, 6), but appreciable hybridization still occurs (4, 7–10) without intrinsic hybrid inviability or sterility (11). Although the aquatic larvae of both forms also may be collected from the same breeding site, S-form larvae are associated with ephemeral and largely predator-free pools of rain water, whereas M-form larvae exploit longer-lived but predator-rich anthropogenic habitats (12). Thus, persistence of M and S despite hybridization may be driven by ecologically dependent fitness trade-offs in the alternative larval habitats to which they are adapting (12).

Under a model of speciation in the presence of gene flow, genomic divergence between incipient species should be limited to regions containing the genes that confer differential adaptations or are involved in reproductive isolation (13). Consistent with this expectation, scans of genomic divergence between M and S at the resolution of gene-based microarrays revealed elevated divergence near the centromeres of all three independently assorting chromosomes, and almost nowhere else (14, 15). Given the assumption of appreciable genetic exchange through hybridization, this pattern suggested that the genes causing ecological and behavioral isolation were located in the centromeric “speciation islands” (14). The small number, size, and gene content of these islands implied that speciation of M and S was very recent and involved only a few genes in a few isolated chromosomal regions—an influential model for speciation with gene flow (13, 16, 17). The complete genome sequences of *A. gambiae* M and S forms reported here provide much higher resolution than previous studies to address how genomes diverge during speciation.

Sequences were determined from colonies established in 2005 from Mali, where the rate of natural M-S hybridization (~1%) is theoretically high enough for introgression to homogenize neutral variation between genomes (18) in the absence of countervailing selection. Both colonies were homosequential and homozygous with respect to all known chromosomal inversions with the exception of 2L_a and 2R_c (19). Independent draft genome assemblies were generated based on ~2.7 million Sanger traces (19). Both assemblies were performed independently of the reference *A. gambiae* PEST genome (20), which is a chimera of the M and S forms. Genome assembly metrics were similar between M and S (table S1) (19). Lower coverage (~6-fold in M/S versus ~10-fold in PEST) contributed to assembly gaps, motivating alignment of the M and S scaffolds to the PEST assembly for transfer of genomic coordinates and gene annotations (www.vectorbase.org; table S2) (19). We confirmed the major trends of M-S divergence by direct alignment of M and S scaffolds to each other (fig. S1) (19).

More than two million single-nucleotide polymorphisms (SNPs) per form and more than 150,000 fixed differences between forms were identified in the sequence data using strict coverage and quality restrictions (table S3) (19). The chromosomes show significantly different patterns of divergence, with chromosome 2 showing proportionally more fixed differences than chromo-

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some 3, and chromosome X showing the highest proportion of fixed differences [further explored in (19)] (table S3). The spatial distribution of polymorphism and divergence along chromosome arms also was investigated, using sliding window analyses to minimize noise from individual site-based divergence estimates (Fig. 1 and figs. S2 to S6) (19, 21). Significant outlier divergence values

falling in the top 1% of the empirical distribution (fig. S7) (19, 22) are spread heterogeneously across the entire genome, not confined mostly to pericentromeric regions as observed in gene-based microarray studies (14, 15, 19).

The 436 genes overlapping with the top percentile of diverged 1-kb windows were tested for functional enrichment based on their gene ontol-

ogy terms (database S1) (19). The 1-kb window size, smaller than the average gene size (~5.7 kb, including introns), mitigates the potentially confounding effect of physical clustering of functionally related members of gene families in *A. gambiae*. Functions related to G-protein-coupled receptor (GPCR) signaling, particularly neurohormone signaling, are significantly overrepresented in genomic regions of highest divergence (table S4). The neurohormone subfamily of GPCRs bind biogenic amines, neuropeptides, and protein hormone ligands, which in insects control development, feeding, reproduction, and complex behaviors (e.g., locomotion) that potentially bear on niche adaptation and mate recognition.

We also examined genes for evidence of divergence. Genes showing evidence of directional selection within forms, or amino acid fixations between forms (database S1 and figs. S2 to S6) (19), occur throughout the genome, suggesting that differential adaptation of M and S to their specific ecologies could involve an appreciable number of genes outside of pericentromeric regions. Some genomic regions appear to have experienced strong and recent selective sweeps, as illustrated by elevated divergence coupled with reductions in shared and private polymorphism (Fig. 1 and figs. S2 to 6). The most notable such region is on 2L (near Mb 25) centered on the *resistance to dieldrin (Rdl)* gene, which has been previously associated with insecticide resistance in *A. gambiae* and other insects (Fig. 1 and fig. S4) (23). In fact, M and S appear to carry different “resistant” substitutions (Ala296Ser in M, Ala296Gly in S) at *Rdl* (23) suggesting independent selective sweeps. Another notable region occurs on 3R near position ~40 Mb (Fig. 1 and fig. S4) and contains seven odorant receptors (ORs) whose closest match to the proteome of the fruit fly *Drosophila melanogaster* is OR67d. The single copy of this gene in *Drosophila* serves as the pheromone receptor for cis-vaccenyl acetate that mediates both social aggregation and female sexual receptivity (24), tempting speculation that these genes might play similar yet species-specific roles in M and S.

The pattern of genome-wide divergence inferred from colony-based genomic sequences is present in natural populations of M and S from the same region of Mali, based on a newly developed SNP genotyping array whose design included a subset of 400,000 SNPs derived from the M and S genome sequences (25). Indeed, visual and statistical concordance of patterns of divergence (fig. S8 and table S5) between the two data sets indicates that, at least in Mali, the widespread genomic divergence observed between M and S is not an artifact of laboratory culture (19). Future genome-wide studies spanning different geographic locations will be necessary to provide insight into whether and how this pattern varies spatially. Further population genomic sequencing by current short-read technologies will benefit from read-mapping to the independent M and S genome assemblies reported here.

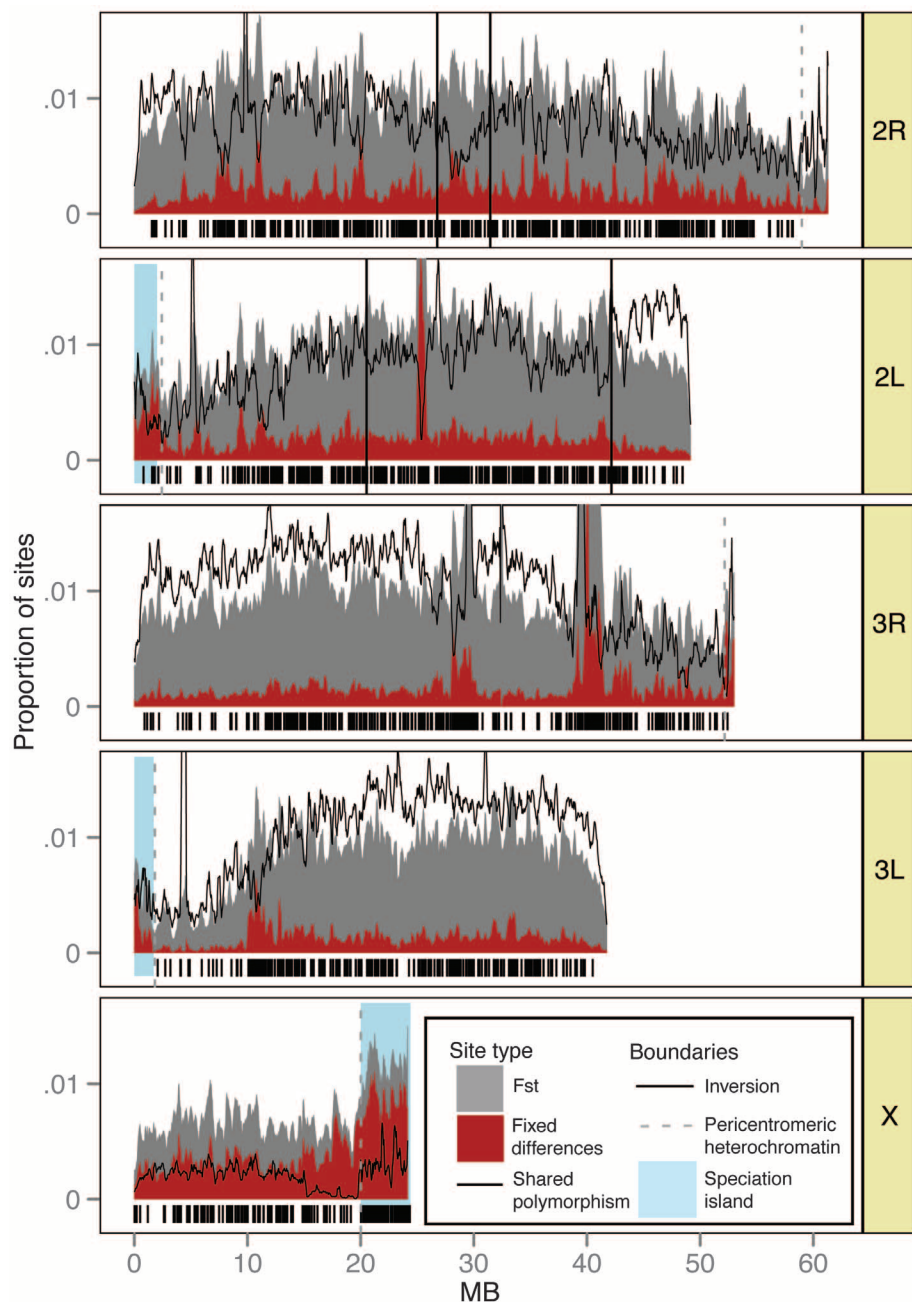


Fig. 1. Sliding window analysis of polymorphism and divergence in M and S based on 250-kb windows with 50-kb steps. Approximate boundaries of chromosomal rearrangements differing between M and S colonies (2Rc and 2La) are indicated by solid black vertical lines. Speciation islands sensu (14, 15) are shaded in blue for reference. F_{ST} refers to the mean per-site estimate (19). Under the x axis, vertical black bars mark the approximate location of 1-kb windows whose divergence values fall in the top percentile of the distribution across autosomes (or the X chromosome, calculated separately). For both 250-kb and 1-kb windows, only windows meeting coverage and quality restrictions (19) are plotted.

The widely adopted model of ongoing speciation-with-gene-flow for M and S (14) posits that frequent hybridization leads to M-S genome homogenization in all except a few small regions near centromeres (“speciation islands”), which are barred from introgression because they contribute to differential fitness (i.e., ecological and reproductive isolation). Detection of much more widespread genomic divergence based on genotyping (25) and whole genome sequencing supports a very different model, in which realized gene flow between forms is currently much lower, and the process of speciation more advanced, than previously recognized, with the corollary that identification of genetic changes instrumental and not merely incidental to their ecological and behavioral divergence will be more difficult than initially hoped. However, powerful resources in the form of independently assembled M and S genomes and a SNP genotyping array (25) are now available for detecting morphologically cryptic vector subdivisions, probing their molecular basis, and ultimately developing innovative malaria interventions.

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Supporting Online Material

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SNP Genotyping Defines Complex Gene-Flow Boundaries Among African Malaria Vector Mosquitoes

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Mosquitoes in the *Anopheles gambiae* complex show rapid ecological and behavioral diversification, traits that promote malaria transmission and complicate vector control efforts. A high-density, genome-wide mosquito SNP-genotyping array allowed mapping of genomic differentiation between populations and species that exhibit varying levels of reproductive isolation. Regions near centromeres or within polymorphic inversions exhibited the greatest genetic divergence, but divergence was also observed elsewhere in the genomes. Signals of natural selection within populations were overrepresented among genomic regions that are differentiated between populations, implying that differentiation is often driven by population-specific selective events. Complex genomic differentiation among speciating vector mosquito populations implies that tools for genome-wide monitoring of population structure will prove useful for the advancement of malaria eradication.

Anopheles gambiae is the primary vector of human malaria in sub-Saharan Africa, where annual burdens of malaria-induced morbidity and mortality are greatest. Population

subdivision within *A. gambiae* is pervasive but has been defined inconsistently and incompletely in the past. *A. gambiae* is composed of at least two morphologically identical incipient species known as the M and S molecular forms based on fixed ribosomal DNA sequence differences (1). The M and S forms are further divided by inversion karyotype into five distinct chromosomal forms, including Mopti (molecular form M), Savanna (molecular form S), and Bamako (molecular form S), each of which we examine here, and each of which has specialized for different breeding sites (2, 3). Furthermore, *A. gambiae* belongs to a species complex of seven recently diverged, morphologically identical sibling taxa, including another major malaria vector, *A. arabiensis*, which we also examine here. Population sub-

division can increase disease transmission intensity and duration, as new mosquito populations evolve to exploit changing habitats and varied seasonal conditions. Vector control efforts can be complicated by population subdivision, because populations vary for traits on which interventions depend, such as indoor feeding behavior (4, 5) and insecticide susceptibility (6).

Genes underlying epidemiologically relevant phenotypic diversification among vector populations must reside within genomic regions that are differentiated among those populations. Most previous efforts to detect genetic differentiation between mosquito populations have been unable to localize differentiated regions, even when population divergence has been detected [for instance, between S and Bamako (7)] or lacked resolution to map all but the most highly differentiated regions [for example, between M and S (8, 9)]. High-resolution mapping of genomic regions differentiated between vector populations will advance our understanding of phenotypic diversification. Furthermore, ongoing assessment of gene flow among vector populations is essential for implementation of control measures designed for natural genetic variants [for instance, insecticide susceptibility alleles (10)] or introduced transgenic variants (11) within mosquito populations, as we strive yet again to eradicate malaria.

We used a customized Affymetrix single-nucleotide polymorphism (SNP) genotyping array to analyze 400,000 SNPs identified through sequencing of the M and S incipient species of *A. gambiae* (12). We hybridized individual arrays with genomic DNA from each of 20 field-collected females from the three known sympatric *A. gambiae* populations in Mali (M, S, and Bamako) that exhibit partial reproductive isolation (2, 13–15). We then hybridized DNA pooled from the same 20 females from each population to determine the

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degree to which quantitative differences in allele frequencies could be assessed with the use of pooled DNA. We also hybridized a pool of DNA from 20 field-collected individuals of the sister species *A. arabiensis*. Results obtained from pooled and individual hybridizations were highly correlated (Pearson's correlation coefficient $r^2 = 0.96$ for M, S, and Bamako comparisons) (fig. S1), indicating that the majority of SNPs assayed on the array yield useful quantitative information regarding divergence in allele frequencies between pooled samples.

Pooled hybridization data revealed that the greatest differentiation between the recently subdivided S and Bamako populations maps within a cluster of inversions on chromosomal arm 2R (Fig. 1A). This pattern is concordant with models of speciation in the face of ongoing gene flow, which predict that early in the speciation process, divergence will be localized to regions of low recombination, such as inversions (16–20). In partially reproductively isolated populations like S and Bamako, these divergent genomic regions are most likely to contain genes (table S2) directly responsible for differential niche adaptation and reproductive isolation, whereas ongoing gene flow should homogenize the remainder of the genome (21–23).

The M and S mosquito populations in Mali exhibit divergence that is much greater and more heterogeneous overall than that observed between S and Bamako (Fig. 1B). This might be expected given the broader geographic ranges of M and S relative to Bamako and their presumed longer divergence time (2). We found that all pericentromeric regions exhibit high levels of differentiation between M and S (fig. S2), in accordance with previous observations (8, 9, 24). However, we unexpectedly detected shorter regions of substantial differentiation at various distances from centromeres along each chromosome. The existence of extensive divergence within nonpericentromeric regions suggests that realized gene flow between these two incipient species is low, despite the observation of hybrids between M and S at frequencies approaching 1% in Mali (25). These findings, which we obtained with the use of DNA isolated from field-collected mosquitoes, reinforce patterns observed in the sequencing-based SNP analysis of M and S mosquito colonies (12).

We also compared the Mali S pool to a pool of colony-derived M mosquitoes from Cameroon to address the possibility that differentiation observed between M and S is geographically restricted to Mali. Genetic differentiation is substantially greater between M populations from Mali and Cameroon than between S populations from these locations, and it has been speculated that another incipient speciation event may be occurring within M (26). However, with the exception of the 2La inversion, we find extremely similar patterns of differentiation between S and M, regardless of the geographic origin of the M population that was analyzed (Fig. 1C and fig. S3). This finding suggests that the genomic regions differentiated

between M and S are probably similar throughout West and Central Africa and may harbor the genes facilitating niche differentiation as well as pre- and postmating isolation between these taxa. However, the great extent of genomic divergence also implies that identifying the genes involved in the earliest stages of the M and S speciation process will prove challenging.

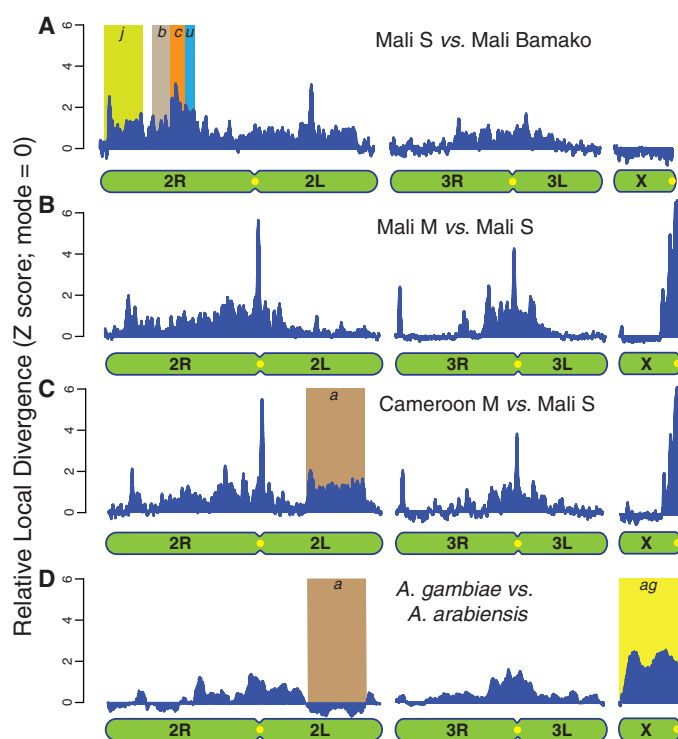
Finally, we compared *A. gambiae* and its sister species *A. arabiensis*, between which hybridization can occur in nature, although it yields sterile males (27). Because SNPs assayed on the array are segregating in *A. gambiae* but may not be segregating in *A. arabiensis*, we could not compare the overall magnitude of genomic divergence between these taxa with the divergence between forms of *A. gambiae*. To avoid bias, we undertook this comparison with a subset (75,750) of the SNPs that were found to exhibit similar allelic intensity ratios in the M and S pools. This assay set was sufficient to indicate that the profile of relative differentiation between *A. gambiae* and *A. arabiensis* is less heterogeneous than that in the M versus S comparison (Fig. 1D), even as it echoes some of the same highly divergent regions. Chromosomes 2 and 3 exhibit slightly heightened pericentromeric differentiation, similar to the pattern we observed between the M and S forms of *A. gambiae*, with additional differentiation across the entire X chromosome, presumably due to the large *Xag* inversion fixed in *A. gambiae* and at least one additional inversion fixed in *A. arabiensis* relative to the ancestral X arrangement (28, 29).

Although particular inversion arrangements are not exclusive to any of the *A. gambiae* populations that we profiled, these genomic regions

clearly harbor an excess of differentiation between populations compared with other regions of the genome (Figs. 1 and 2). Inversions may be hotspots for differentiation, even when maintained at similar frequencies in different populations, if recombination suppression facilitates functional divergence of the inverted and wild-type arrangements. Average linkage disequilibrium within all three forms of *A. gambiae* is extremely low, extending no more than a few thousand base pairs (fig. S4). Therefore, groups of loci that reside within regions of lower recombination in the *A. gambiae* genome would be more likely to establish consistent patterns of cosegregation.

It is important to distinguish a difference in inversion frequency between populations versus differentiation of alternative inversion arrangements between populations. Principal components analysis (PCA) of SNP genotypes within inversion boundaries indicates that, although the S and Bamako populations harbor different frequencies of the 2Rj, 2Rb, 2Rc, and 2Ru inversions (table S3), the *b* arrangement of 2Rb is divergent between S and Bamako (Fig. 2). This result indicates that, although this arrangement is frequent in both S and Bamako, it is differentiating independently within each population. Similarly, both arrangements of 2La and 2Ru, have differentiated between M and S (Fig. 2 and table S3). However, the inverted 2La arrangement is an exception to this pattern: M, S, and Bamako mosquitoes homozygous for the 2La inversion exhibit much less divergence between the 2La breakpoints than is observed in the same three populations for all other inversions (Fig. 2A). The close clustering of

Fig. 1. Relative local divergence profiles for pairwise comparisons of mosquito populations, represented by z scores (standard deviations) and scaled so that 0 reflects the modal divergence for each comparison. Plots represent average difference in allelic intensity ratios measured over adjacent 50 SNP stepping windows. The colored regions labeled with letters represent chromosomal inversion locations. (A) Divergence between S and Bamako forms of *A. gambiae* from Mali. (B) Divergence between *A. gambiae* M-form mosquitoes and S-form mosquitoes from Mali. (C) Divergence between M-form mosquitoes from Cameroon and S-form mosquitoes from Mali. (D) Divergence between *A. arabiensis* from Burkina Faso and *A. gambiae* from Mali.



A. arabiensis (a species fixed for the inverted 2La arrangement) with individuals homozygous for 2La from each of the *A. gambiae* populations (M, S, and Bamako) supports earlier hypotheses regarding introgression between species within this region (30). Indeed, the region within the 2La inversion breakpoints shows divergence between *A. gambiae* and *A. arabiensis* that is lower than expected (Fig. 1D). Overall, these PCA plots highlight the degree of similarity within each of these partially isolated populations. With the exception of 2Rj, no inversion is diagnostic of a particular population in our sample. However, the consistently independent clustering of M, S, and Bamako mosquitoes by PCA across all inversions except 2La affirms the legitimacy and genetic distinctiveness of these groups.

We next examined the data for signals of natural selection. The genomic regions exhibiting greatest divergence ($F_{st} > 0.6$) between M and S exhibit significantly reduced polymorphism in one or both species (fig. S5) [M: one-tailed t test, $P = 1.14 \times 10^{-47}$ (1.14E-47); S: one-tailed t test, $P = 1.88\text{E-}120$], as might be expected if differences between populations were driven to fixation by polymorphism-eliminating selective sweeps (31). To explore selection more deeply, we analyzed SNP calls from individual hybridizations of M, S, and Bamako mosquitoes with the use of SweepFinder software (32), an approach that evaluates the likelihood of a sweep within a particular genomic region, given the allele frequency spectrum of local SNPs. Several genomic regions appear to have experienced recent sweeps within each of the three forms (Fig. 3). The pericentromeric regions of all three chromosomes exhibit the strongest signals of selective sweeps for M and S, suggesting that the extensive divergence observed in these regions has been driven by selection (Fig. 3). Indeed, the degree of concordance between the profiles of selection and differentiation for M and S [chi-squared test, $P = 2.4\text{E-}104$ (14)] implies a causal role for selection within some genomic regions where differentiation is observed. Additionally, the fact that different regions of the genomes of M, S, and Bamako show evidence of selective sweeps suggests that these populations are experiencing different selective pressures that shape genetic variation independently (Fig. 3).

Analysis for functional enrichment (15) among 68 genes found in candidate sweep regions identified two interesting categories of genes significantly overrepresented after correction for multiple testing: (i) multicellular organismal development ($P = 9.1\text{E-}4$; five genes), and (ii) serine-type endopeptidase activity ($P = 2.6\text{E-}2$; nine genes, five of which occur in a pericentromeric cluster on 3L). Of the five genes annotated as being involved in development, three encode homeodomain-containing transcriptional regulators (AGAP004659, sweep in M; AGAP004660, sweep in S; AGAP004696, sweep in Bamako), one encodes a member of the Hedgehog signaling pathway (AGAP004637, sweep in S), and one

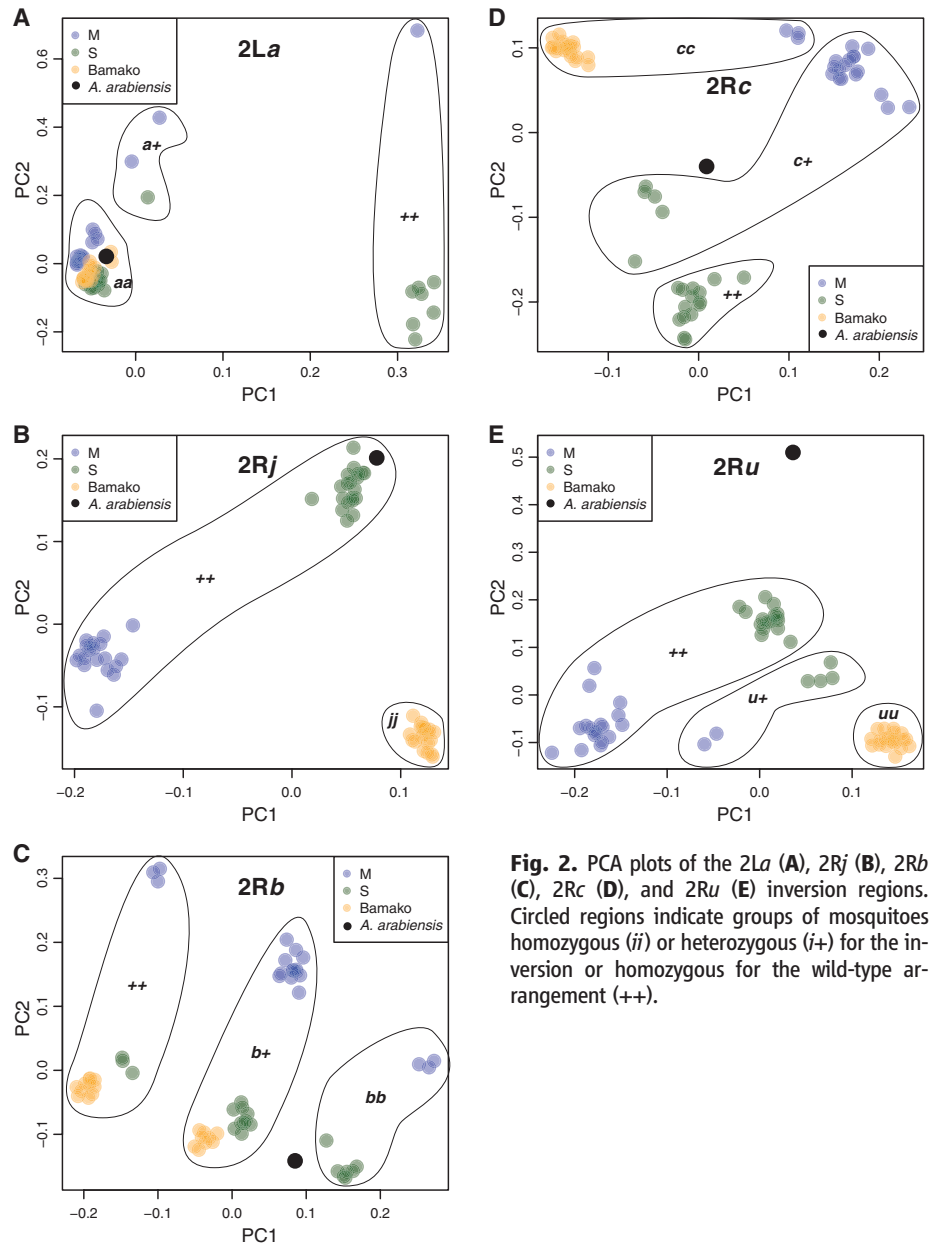


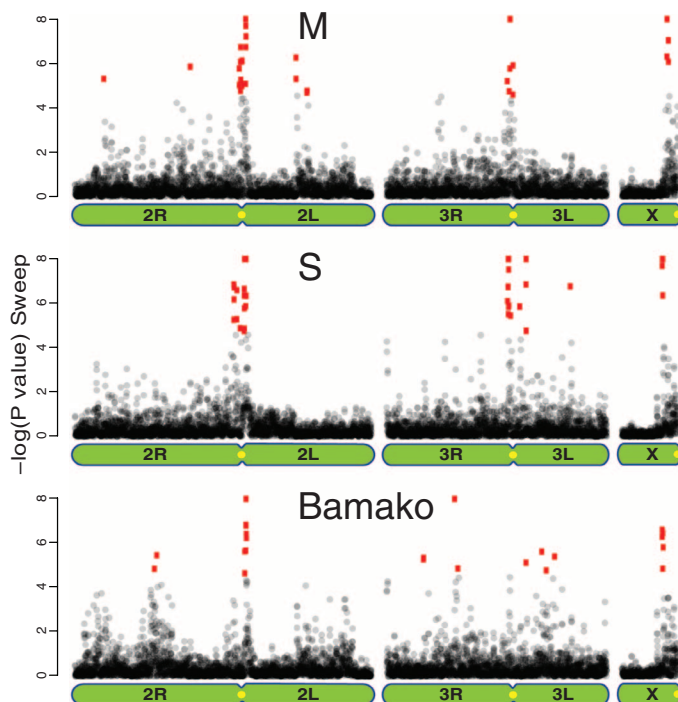
Fig. 2. PCA plots of the 2La (A), 2Rj (B), 2Rb (C), 2Rc (D), and 2Ru (E) inversion regions. Circled regions indicate groups of mosquitoes homozygous (ii) or heterozygous (i+) for the inversion or homozygous for the wild-type arrangement (++).

encodes a member of the Wnt signaling pathway (AGAP010283, sweep in M), indicating that shifts in developmental regulatory programs may underlie ecological niche differentiation and/or reproductive isolation mechanisms that reinforce the ongoing process of speciation in these populations. The gene encoding CPF3, a cuticular protein speculated to bind sex pheromones (33), is also found in a pericentromeric sweep region in S on chromosome 2L. *CPF3* is the gene exhibiting the most significant difference in expression between M and S (33), and it dramatically changes expression upon mating (34). These combined observations motivate further investigation of *CPF3* and its potential relation to M and S mate discrimination. Table S2 presents a full list of the 536 genes found in differentiated and/or sweep regions. Among this set of 536 loci, genes from the X chromosome

are significantly overrepresented (X total = 173; chi-squared test; $P < 2.2\text{E-}16$).

Our findings demonstrate the power of high-resolution SNP arrays for mapping genetic divergence among vector mosquito taxa within the *A. gambiae* species complex. The ability to detect differentiation between distinct populations and selective sweeps within populations is valuable for identifying and monitoring alleles that mediate traits critical for malaria transmission and vector control. The differentiated genomic regions we have identified with these comparisons harbor genes (table S2) of epidemiological importance for disease transmission, including loci influencing reproduction, longevity, insecticide resistance, aridity tolerance, larval habitat, and other traits that differ among mosquito populations (2).

Fig. 3. Profiles of genomic regions subject to recent selective sweeps in M, S, and Bamako forms of *A. gambiae*. Each point represents the $-\log P$ value of a selective sweep for a window of ~ 20 SNPs. Windows exhibiting significant signals of selection ($P < 0.05$ after Bonferroni correction) are indicated in red.



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Supporting Online Material

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dbSNP Accession Numbers

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ATM Activation by Oxidative Stress

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The ataxia-telangiectasia mutated (ATM) protein kinase is activated by DNA double-strand breaks (DSBs) through the Mre11-Rad50-Nbs1 (MRN) DNA repair complex and orchestrates signaling cascades that initiate the DNA damage response. Cells lacking ATM are also hypersensitive to insults other than DSBs, particularly oxidative stress. We show that oxidation of ATM directly induces ATM activation in the absence of DNA DSBs and the MRN complex. The oxidized form of ATM is a disulfide-cross-linked dimer, and mutation of a critical cysteine residue involved in disulfide bond formation specifically blocked activation through the oxidation pathway. Identification of this pathway explains observations of ATM activation under conditions of oxidative stress and shows that ATM is an important sensor of reactive oxygen species in human cells.

Patients with ataxia-telangiectasia (A-T) lack functional A-T mutated (ATM) protein and exhibit a pleiotropic phenotype that includes cerebellar ataxia, immunodeficiency, premature aging, and a high incidence of lymphoma (1). These defects may be connected through dysfunctional control of reactive oxygen species (ROS), indicated by observations that mammalian cells

lacking ATM exhibit high concentrations of ROS and hypersensitivity to agents that induce oxidative stress (2). Lymphoma incidence and the loss of hematopoietic stem cells that occurs in mice lacking ATM can be suppressed by antioxidants (3–5), indicating an important role for ATM in regulating cellular defenses against redox stress. ATM is activated in response to changes in in-

tracellular redox status (6–10), but it has not been clear what the initiating event is in these cases or how this relates to Mre11-Rad50-Nbs1 (MRN)-mediated ATM activation that is dependent on DNA double-strand breaks (DSBs).

To address these questions, we used primary human fibroblasts and induced oxidative stress with H₂O₂ or DSBs with bleomycin (11). Autophosphorylation of ATM on Ser¹⁹⁸¹, phosphorylation of the tumor suppressor p53 on Ser¹⁵, and phosphorylation of the protein kinase Chk2 on Thr⁶⁸ all occurred in response to H₂O₂ or to bleomycin treatment (Fig. 1, A to C). Phospho-

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rylation of histone H2AX (γ -H2AX), a marker for DNA DSBs, only occurred after bleomycin treatment (Fig. 1D), indicating that ATM activation induced by H_2O_2 can occur in the absence of DNA damage. Like histone H2AX, the heterochromatin protein Kap1 was phosphorylated at Ser⁸²⁴ after exposure of cells to bleomycin but not after H_2O_2 treatment (Fig. 1E), indicating that only a subset of ATM targets that are phosphorylated during the DNA damage response are also phosphorylated during oxidative stress. Treatment of cells with an ATM-specific inhibitor (Ku-55933) blocked phosphorylation of ATM, p53, and Chk2 during subsequent exposure to H_2O_2 (Fig. 1, F to H), confirming that they are in fact ATM-dependent. Similar results were also observed in cultured human embryonic kidney (HEK) 293T cells (fig. S1). Our results show a correlation between oxidative stress and phosphorylation of substrates that are not stably associated with DNA (p53 and Chk2), whereas DNA-associated substrates (H2AX and Kap1) are specifically targeted in response to DNA damage when ATM is recruited to DNA by MRN (12, 13).

Patients with ataxia-telangiectasia-like disorder (ATLD) express a mutated form of Mre11 and are impaired in activation of ATM through DSBs (12, 14). To test for the role of MRN in ATM activation by oxidative stress, we used fibroblasts established from an ATLD patient and complemented with either green fluorescent protein (GFP) as a negative control or wild-type Mre11. Expression of wild-type Mre11 in these cells restores normal amounts of functional MRN complex to the nucleus (15). p53 and Chk2 were both phosphorylated by ATM in ATLD cells and in

Mre11-complemented cells that were exposed to H_2O_2 (Fig. 1, I to L). Thus, MRN is not essential for activation of ATM by oxidation, and ATM activation through this pathway may be separate from the DNA damage response machinery.

Dimeric ATM purified from mammalian cells is completely inactive but is strongly activated by addition of recombinant MRN complex and DNA ends in a purified protein system in vitro (13). We tested whether oxidative stress could also activate ATM in vitro. The addition of H_2O_2 to purified dimeric ATM (fig. S2) stimulated its activity toward a p53 substrate [the N terminus of p53 fused to glutathione S-transferase (GST)] to an extent similar to those observed with MRN and DNA (Fig. 2, A and B). ATM activation by H_2O_2 in vitro was completely inhibited by the reducing reagent N-acetyl-cysteine (NAC), whereas NAC had little or no effect on the stimulation by MRN and DNA (Fig. 2B). Autophosphorylation of ATM appeared not to be essential for H_2O_2 -mediated activation of ATM, because full activity was observed with the Ser¹⁹⁸¹ $\rightarrow$ Ala¹⁹⁸¹ (S1981A) autophosphorylation site mutant (Fig. 2C). We store recombinant ATM in 1 mM dithiothreitol (DTT) because it exhibits spontaneous activation if purified and stored in the absence of reducing agents (fig. S3), so the effective concentration of H_2O_2 in the in vitro reactions is lower than cited here.

Association of the MRN complex with ATM results in an increase in the affinity of ATM for its substrates (13). To test the mechanism of H_2O_2 activation, we investigated substrate binding by immobilizing ATM on magnetic beads, incubating the beads with a substrate (GST-p53) and quantifying the amount of substrate bound. Oxi-

dation of ATM by H_2O_2 increased the affinity of ATM for GST-p53 (Fig. 2D). H_2O_2 -treated ATM also bound ATP more efficiently (fig. S4). Both results indicate that conformational changes occur in ATM upon oxidation.

In mammalian cells, ATM is initially an inactive, noncovalently associated dimer but converts to an active monomer upon DNA damage (16). With H_2O_2 treatment in vitro, however, ATM instead formed covalent dimers in denaturing SDS-polyacrylamide gel electrophoresis (PAGE) gels that were sensitive to reducing agents (Fig. 2E). A similar pattern of ATM dimer formation was also seen with ATM immunoprecipitated from human cells exposed to H_2O_2 and probed with antibodies directed against ATM or ATM phosphorylated at Ser¹⁹⁸¹ (Fig. 2F). The autophosphorylated ATM seen after H_2O_2 treatment was exclusively in the dimer state, indicating that the activated ATM did not undergo a dimer-to-monomer transition, as it does in response to DNA damage (16). Glycerol gradient ultracentrifugation of purified ATM treated with H_2O_2 in vitro confirmed that oxidized ATM migrated with the apparent molecular size of an ATM dimer (Fig. 2G).

These results led us to test whether one or more intermolecular disulfide bonds form between ATM monomers during oxidative stress and whether these are important for the activation of ATM. Disulfide bonds are reversible by reducing agents, whereas some other forms of oxidized cysteine are not. We therefore exposed purified ATM to H_2O_2 , then incubated it with NAC, and then measured activity. Activation was strongly inhibited by NAC (Fig. 2H), consistent with disulfide bonds being required for activation by H_2O_2 .

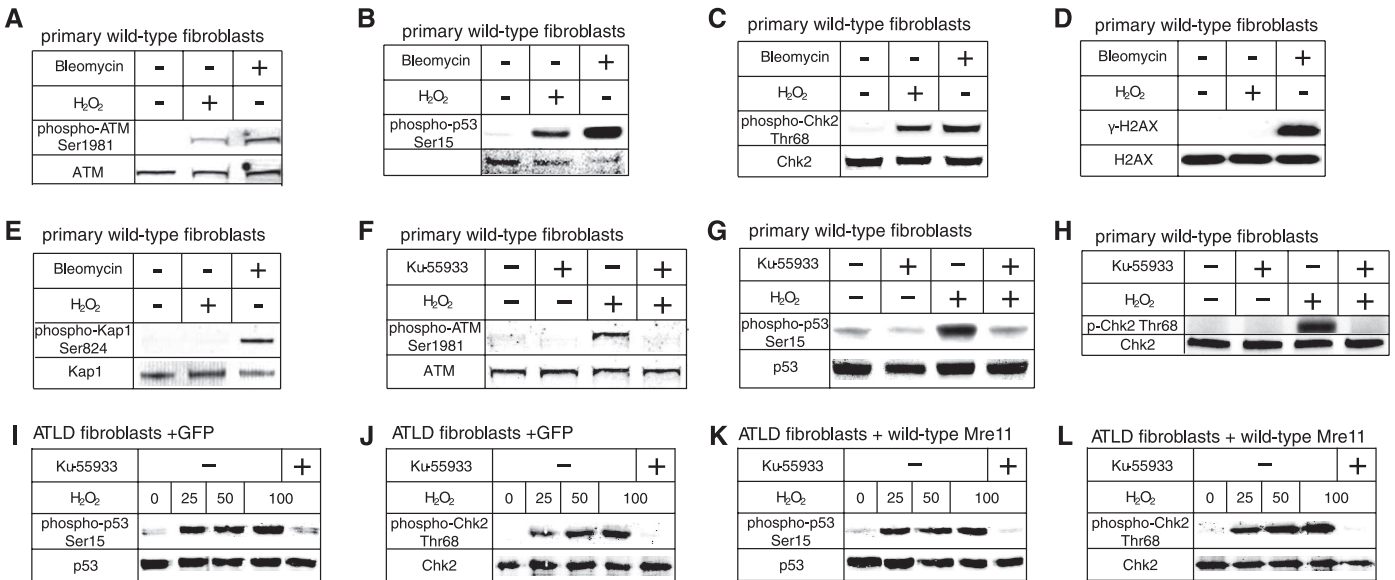


Fig. 1. Activation of ATM by H_2O_2 in the absence of DNA DSBs. (A to E) Human primary fibroblasts (GM08399B) were treated with 250 μ M H_2O_2 or 10 μ g/ml bleomycin for 30 min. Proteins from cell lysates were analyzed by SDS-PAGE and Western blotting for phospho-ATM Ser¹⁹⁸¹, phospho-p53 Ser¹⁵, phospho-Chk2 Thr⁶⁸, phospho-histone H2AX Ser¹⁴⁰, phospho-Kap1 Ser⁸²⁴, or the nonphosphorylated proteins as indicated. (F to H) Fibroblasts were treated

with the ATM inhibitor Ku-55933 for 1 hour before the addition of H_2O_2 and assayed for ATM phosphorylation events as in (A). (I to L) ATLD fibroblasts expressing GFP [(I) and (J)] or wild-type Mre11 [(K) and (L)] were treated with H_2O_2 as indicated (25, 50, or 100 μ M). Phospho-p53 Ser¹⁵ and phospho-Chk2 Thr⁶⁸ induced by H_2O_2 were compared with untreated and ATM inhibitor-treated samples and analyzed for ATM phosphorylation events as in (A).

Disulfide bond formation can also be catalyzed by agents that do not generate ROS. For instance, the thiol oxidant diamide can act as a hydrogen acceptor for reactive thiol groups and promotes formation of disulfide bonds between closely opposed cysteine residues (17). Diamide does not form oxygen radicals and is effective as a thiol oxidant under hypoxic conditions (18, 19). ATM-mediated phosphorylation of p53 in vitro was stimulated by diamide (Fig. 2I). ATM autophosphorylation and phosphorylation of p53 on Ser¹⁵ were both increased after treatment of human cells with diamide (Fig. 2J), indicating that disulfide bond formation is very likely an essential component of ATM activation by oxidative stress.

To generate an ATM mutant deficient in the oxidation pathway, we made several mutations in cysteine residues in the ATM protein on the basis of conservation, association with cancer or A-T, and mass spectrometry-based analysis of ATM disulfide bonds (table S1), but mutation of these sites did not block ATM activation by oxidation. However, mutation of the only cysteine in the

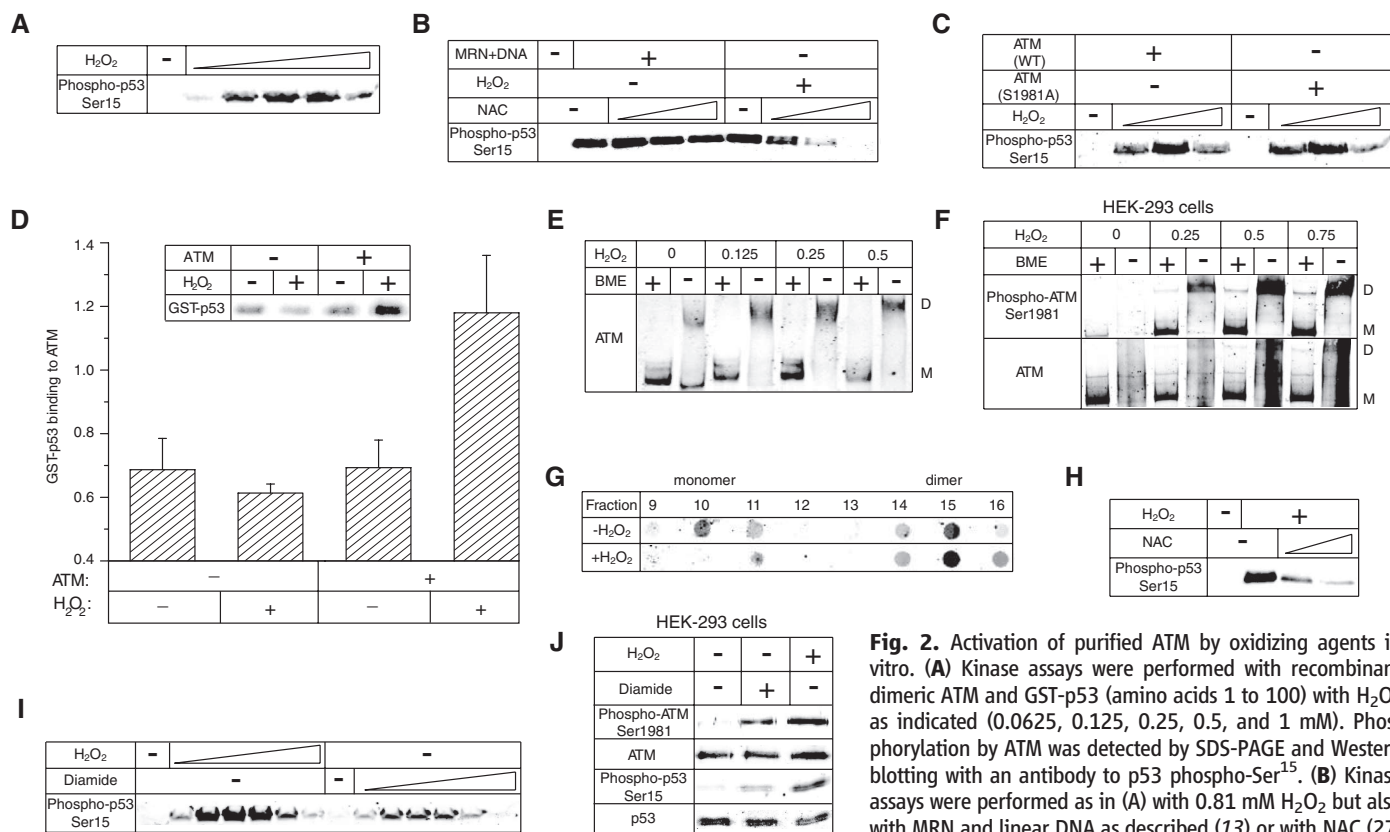
FRAP/ATM/TRRAP C-terminal (FATC) domain, Cys²⁹⁹¹, resulted in an ATM mutant that was fully activated by MRN and DNA in vitro but not by H₂O₂ (Fig. 3A). Cys²⁹⁹¹ is located close to the kinase domain of ATM and is conserved among all terrestrial vertebrates, but is not present in ATM from zebrafish or lower eukaryotes (fig. S5).

To determine whether Cys²⁹⁹¹ is involved in disulfide bond formation during ATM oxidation, we treated ATM in vitro with H₂O₂, separated the oxidized ATM by SDS-PAGE, blocked all reactive thiol groups with iodoacetamide, then reduced the thiols in disulfides with Tris(2-carboxyethyl) phosphine (TCEP), a disulfide-specific reducing agent. The thiols reduced by TCEP were modified with N-ethyl maleimide (NEM), and the protein was digested with trypsin. Analysis of the tryptic peptides by mass spectrometry showed that the peptide containing Cys²⁹⁹¹ reacted with acrylamide in the gel to generate a propionamide adduct in the absence of oxidation, consistent with this residue being highly reactive. With ox-

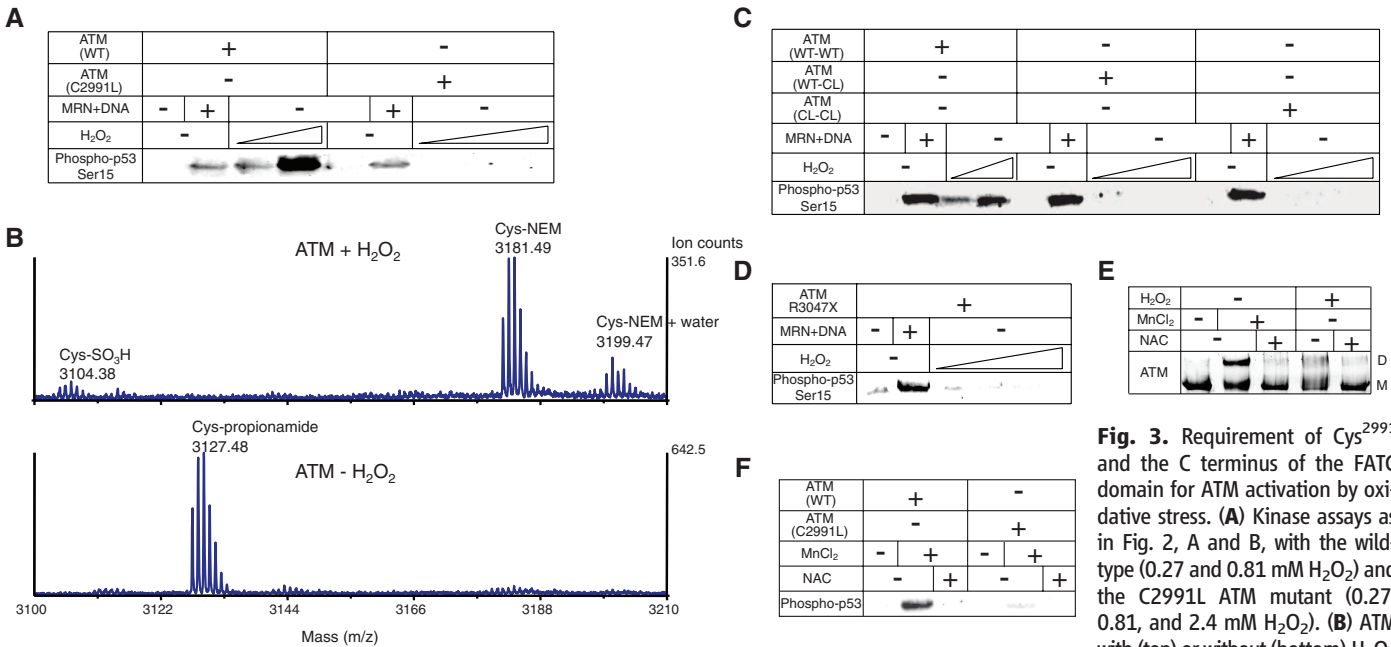
idation treatment, the peptide shows addition of NEM, confirming that Cys²⁹⁹¹ does form a disulfide bond during ATM oxidation (Fig. 3B and table S2).

The Cys²⁹⁹¹ → Leu²⁹⁹¹ (C2991L) mutant still formed a disulfide-cross-linked dimer during H₂O₂ exposure, similar to the wild-type protein, even though it is not active (fig. S6), consistent with our observation that oxidized ATM contains at least several disulfide bonds (table S1). To determine whether Cys²⁹⁹¹ is required in both subunits of the dimer, we prepared heterodimers of ATM containing one wild-type subunit and one C2991L subunit by using two different epitope tags and sequential affinity purification (20). The C2991L-wild-type heterodimeric ATM, like the C2991L homodimeric form, showed a normal response to MRN and DNA but was not activated by H₂O₂ in vitro (Fig. 3C). This result suggests that the disulfide bond formed at Cys²⁹⁹¹ is intermolecular.

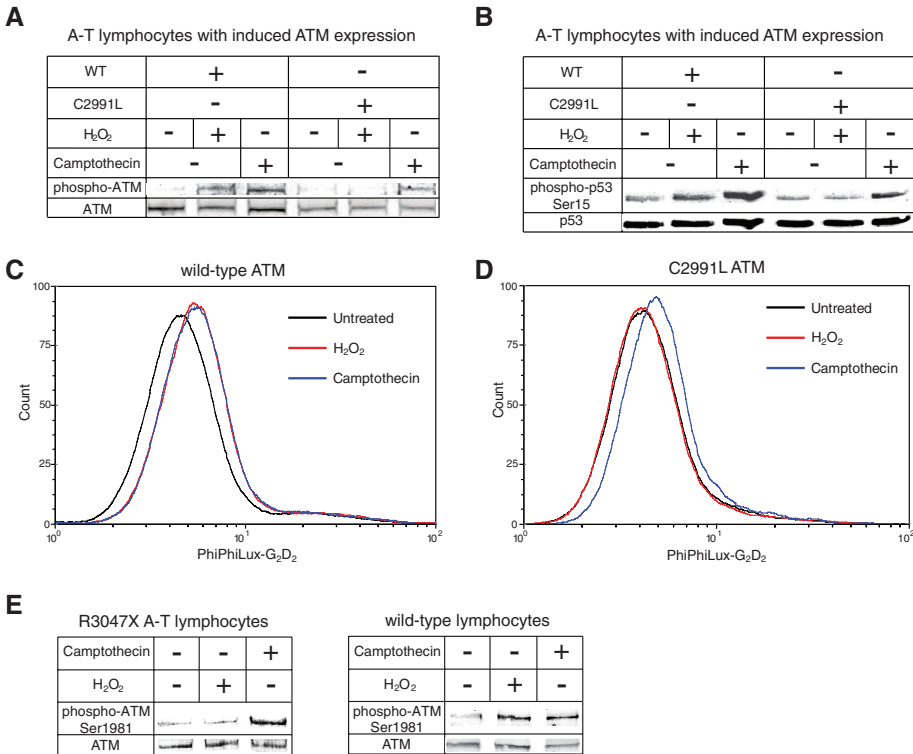
Deletion of the last 10 amino acids of ATM results in a mutant protein (R3047X) that causes A-T; however, patients with this mutation have



or S1981A dimeric ATM and H₂O₂ (0.81, 2.4, and 7.3 mM). (D) Biotinylated ATM was bound to streptavidin beads and incubated with GST-p53 in the presence or absence of H₂O₂ (0.27 mM). The amount of GST-p53 substrate bound was quantified with Western blotting using an antibody to GST. The average of three independent experiments is shown, with error bars indicating standard deviation. (E) ATM oxidized with H₂O₂ in vitro was analyzed by SDS-PAGE in the presence or absence of betamercaptoethanol (BME) and analyzed by Western blotting with an antibody to ATM. M and D indicate the positions of a 350-kD monomer and a 700-kD dimer, respectively. (F) ATM oxidized with H₂O₂ in human 293 cells was immunoprecipitated and analyzed as in (E) with ATM and phospho-ATM Ser¹⁹⁸¹ antibodies. (G) ATM oxidized in vitro was separated by glycerol gradient and visualized by Western blotting in comparison with untreated ATM. Positions of monomer and dimer ATM relative to molecular weight standards are shown. (H) Kinase assays with dimeric ATM in vitro as in (A) with 0.81 mM H₂O₂ except that NAC (0.25 or 0.5 mM) was added before incubation with substrate. (I) Kinase assays as in (A) except with H₂O₂ (0.09, 0.27, 0.8, 2.4, 7.3, or 22 mM) and diamide (0.9, 2.7, 8.3, 25, 75, or 225 μM). (J) Human cells stably expressing wild-type ATM were treated with 0.5 mM diamide for 30 min and analyzed by Western blotting for phospho-ATM Ser¹⁹⁸¹, phospho-p53 Ser¹⁵, ATM, or p53.



was analyzed for disulfide bond formation at the Cys²⁹⁹¹ site as described in the text and (11). The 3181.49 peak shows NEM modification of the Cys²⁹⁹¹-containing peptide in the treated sample; the control is modified by propionamide. (C) Kinase assays as in (A) except with ATM wild-type homodimer (WT-WT), C2991L-WT heterodimer (WT-CL), or C2991L homodimer (CL-CL) proteins with H₂O₂ (0.27 or 0.81 mM for WT and 0.27, 0.81, or 2.43 mM for WT-CL and CL-CL). (D) Kinase assays as in (A) except with the ATM mutant R3047X. (E) ATM dimerization assays as in Fig. 2E, except with 5 mM MnCl₂ or H₂O₂ (0.27 mM). M and D indicate positions of ATM monomer and dimer, respectively. (F) Kinase assays as in (A) except with the C2991L ATM mutant, MnCl₂ (5 mM), and 0.25 mM NAC.



been classified as A-T variants because they do not exhibit immunodeficiency and show reduced radiosensitivity compared with most A-T patients (21–23). The recombinant R3047X ATM exhibits properties similar to that of the C2991L mutant in that MRN/DNA stimulation is normal but activation by oxidation is completely deficient

(Fig. 3D). This region of the FATC domain has also been suggested to be a peroxisome targeting signal, and ATM has been reported to localize with the peroxisome (24).

MnCl₂ has been widely used to activate immunoprecipitated ATM in vitro in the absence of MRN (25–29), but the mechanism of this ac-

Fig. 4. Oxidative activation of ATM in human cells is blocked by the C2991L and R3047X mutations. (A and B) AT1-ABR human lymphocytes inducibly expressing WT or C2991L alleles of ATM were treated with H₂O₂ (25 μM) or camptothecin (10 μg/ml) as indicated. Cell lysates were analyzed by SDS-PAGE and Western blotting for phospho-ATM Ser¹⁹⁸¹, phospho-p53 Ser¹⁵, or the nonphosphorylated proteins as indicated. (C and D) AT1-ABR cells expressing WT or C2991L ATM were treated with H₂O₂ (25 μM) or camptothecin (5 μg/ml). The increase of caspase 3 activity during apoptosis was measured by using the fluorescent indicator PhiPhiLux-G₂D₂ (Oncolmmunin, Incorporated, Gaithersburg, MD). (E) WT or R3047X A-T lymphocytes were treated with H₂O₂ (12.5 μM) or camptothecin (10 μg/ml). Cell lysates were analyzed by SDS-PAGE and Western blotting for phospho-ATM Ser¹⁹⁸¹, phospho-Chk2 Thr⁶⁸, or the nonphosphorylated proteins as indicated.

tivation is not understood. We found that MnCl₂ induced ATM cross-linking in vitro and that MnCl₂-dependent activation was inhibited by the reducing reagent NAC (Fig. 3, E and F). The ATM C2991L mutant showed reduced activation by manganese compared with that of wild-type ATM (Fig. 3F). Thus, MnCl₂ appears to primar-

ily activate ATM through the oxidation pathway. This may be due to trace amounts of Mn^{3+} in preparations of $MnCl_2$ (30).

To further investigate the functional effects of the C2991L mutation, we used lymphoblasts from an A-T patient lacking functional ATM and stably complemented these cells with either wild-type or C2991L alleles of ATM under the control of an inducible promoter (fig. S7). After induction, cells were exposed to a low concentration of H_2O_2 or to camptothecin, a topoisomerase poison that induces DNA breaks. Consistent with the results with purified proteins in vitro, wild-type ATM responded to both H_2O_2 and camptothecin, whereas the mutant allele only responded to camptothecin treatment (Fig. 4, A and B). To determine whether these phosphorylation events affected cell survival, we monitored both groups of cells for apoptosis, which is induced by ROS or DNA damage in lymphocytes (31). The cells expressing wild-type ATM showed a strong apoptotic response to both H_2O_2 and camptothecin (as measured by a fluorescent caspase 3 substrate assay), whereas cells expressing the mutant allele only underwent caspase activation in response to camptothecin (Fig. 4, C and D). Confirmation of these results was also obtained by using propidium iodide staining and annexin V to measure cleavage of nuclear DNA and loss of membrane integrity during apoptosis (figs. S8 and S9).

Considering that C2991L and wild-type heterodimers are inactive in vitro (Fig. 3C), we also overexpressed either the C2991L or a wild-type allele of ATM in cells expressing endogenous wild-type ATM (fig. S10). The cells were treated with bleomycin or H_2O_2 , and phosphorylation of p53 on Ser¹⁵ and Chk2 on Thr⁶⁸ was quantified. Cells overexpressing wild-type ATM showed higher phosphorylation of both p53 and Chk2 in response to H_2O_2 compared with cells overexpressing C2991L ATM; thus, ectopic expression of the C2991L mutant acted as a dominant negative and inhibited the oxidative activation of wild-type ATM in human cells.

Immortalized lymphoblasts derived from an A-T patient expressing the R3047X ATM allele were also analyzed for responses to H_2O_2 and DNA damage, which showed that R3047X ATM failed to autophosphorylate ATM or phosphorylate Chk2 after exposure to H_2O_2 but showed a response to camptothecin that was similar to that seen in cells expressing wild-type ATM (Fig. 4 and fig. S10). It is difficult to make quantitative comparisons because the WT and mutant cells were derived from different individuals. The small decrease in responsiveness of the mutant cells to camptothecin could mean that the reduced response to H_2O_2 also reflects a deficit in response to DSBs, but we interpret the result to show a specific deficit in the oxidative response of the R3047X mutant.

We identified and characterized a pathway of ATM activation that is separate from the previously defined pathway that depends on MRN and DNA ends. ATM appears to act as a redox

sensor in human cells, and, given the large number of substrates identified as ATM targets after DNA damage (32), ATM may similarly regulate global cellular responses to oxidative stress. The observation that the R3047X mutation generates an ataxia phenotype in A-T patients but retains normal activation in response to DNA damage suggests that most of the clinical manifestations of A-T may result from an inability to effectively regulate ROS, an observation that has important consequences for A-T treatment strategies.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6003/517/DC1
Materials and Methods
Figs. S1 to S12
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References and Notes

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The Ligase PIAS1 Restricts Natural Regulatory T Cell Differentiation by Epigenetic Repression

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CD4⁺Foxp3⁺ regulatory T (T_{reg}) cells are important for maintaining immune tolerance. Understanding the molecular mechanism that regulates T_{reg} differentiation will facilitate the development of effective therapeutic strategies against autoimmune diseases. We report here that the SUMO E3 ligase PIAS1 restricts the differentiation of natural T_{reg} cells by maintaining a repressive chromatin state of the *Foxp3* promoter. PIAS1 acts by binding to the *Foxp3* promoter to recruit DNA methyltransferases and heterochromatin protein 1 for epigenetic modifications. *Pias1* deletion caused promoter demethylation, reduced histone H3 methylation at Lys⁹, and enhanced promoter accessibility. Consistently, *Pias1*^{-/-} mice displayed an increased natural T_{reg} cell population and were resistant to the development of experimental autoimmune encephalomyelitis. Our studies have identified an epigenetic mechanism that negatively regulates the differentiation of natural T_{reg} cells.

Naturally occurring thymus-derived regulatory T (nT_{reg}) cells play a critical role in the maintenance of self-tolerance and ho-

meostasis within the immune system (1–4). The transcription factor Foxp3 controls the development and function of T_{reg} cells (5–7). Several regulatory DNA elements within the *Foxp3* locus have been suggested to modulate Foxp3 expression, including the promoter region and conserved noncoding sequences (6–9). Although the transcription factors that positively regulate Foxp3 expression are well characterized (10–13), little is known about the negative regulation of Foxp3.

PIAS1 (protein inhibitor of the activated signal transducer and activator of transcription STAT1)

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Fig. 1. Enhanced $CD4^{+}$ Foxp3 $^{+}$ T $_{reg}$ differentiation in $Pias1^{-/-}$ mice. (A) Western blot analysis of whole-cell extracts from freshly isolated thymocytes and splenocytes with an antibody specific for Ser 90 -phosphorylated PIAS1, total PIAS1, or tubulin. (B) Cells from thymus or spleen of male ($n = 7$) wild-type and $Pias1^{-/-}$ littermates were analyzed by flow cytometry to determine the percentage and the absolute cell numbers of Foxp3 $^{+}$ CD4 $^{+}$ cells (gated on a CD4 $^{+}$ CD8 $^{-}$ population). Similar results were obtained with female mice. (C) Bone marrow was isolated from wild-type and $Pias1^{-/-}$ littermates and injected into the sublethally irradiated $Rag1^{-/-}$ recipient mice ($n = 8$). The thymic CD4 $^{+}$ Foxp3 $^{+}$ population was analyzed 4 weeks after reconstitution by flow cytometry. (D) Same as in (C) except that $Pias1^{-/-}$ or wild-type bone marrow (CD45.2) was mixed with wild-type C57S1JL bone marrow (CD45.1) and injected into the $Rag1^{-/-}$ mice ($n = 3$ for wild-type and $n = 4$ for $Pias1^{-/-}$). Experiments in (A) to (D) were performed at least three times ($n = 3$ to 8 for each experiment). P value was determined by unpaired t test.

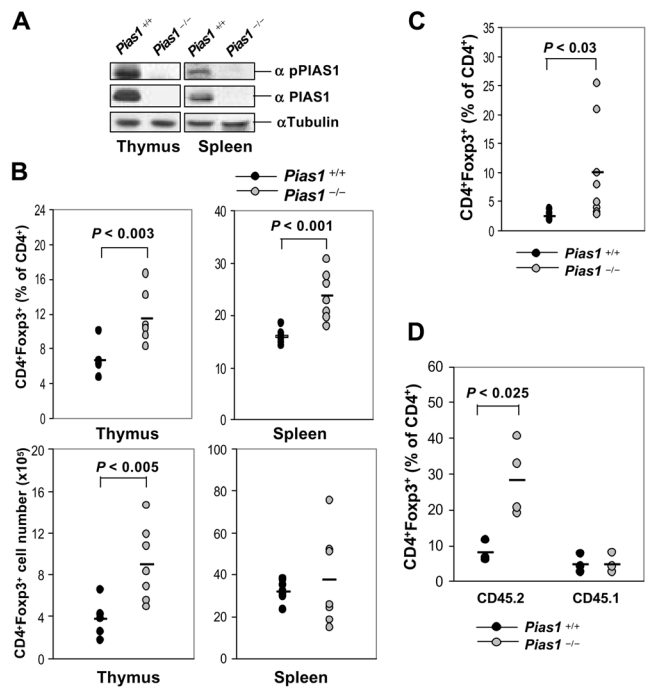
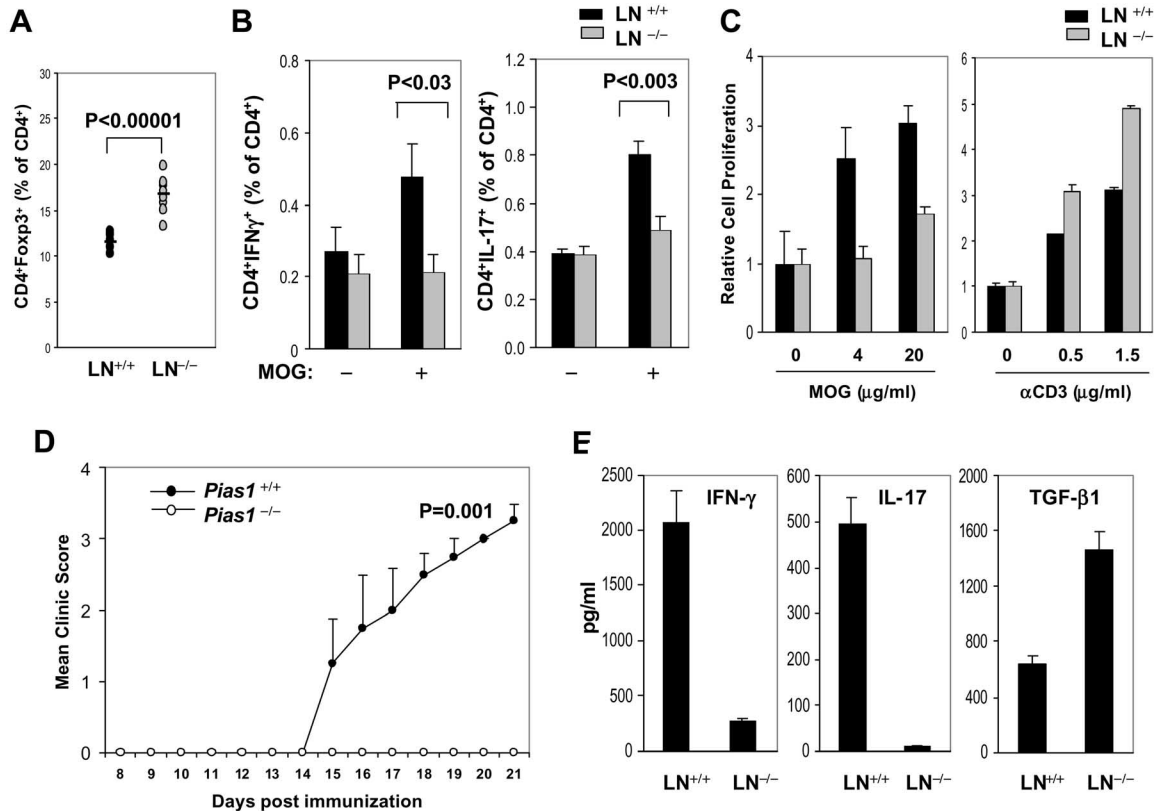


Fig. 2. $Pias1^{-/-}$ mice are resistant to MOG-induced EAE. (A) The percentage of CD4 $^{+}$ Foxp3 $^{+}$ cells in wild-type (LN $^{+/+}$) and $Pias1^{-/-}$ (LN $^{-/-}$) lymph node cells 10 days after a single MOG $_{35-55}$ injection emulsified in Freund's complete adjuvant ($n = 7$). (B) Lymphocytes from $Pias1^{-/-}$ mice and wild-type littermates 10 days after MOG $_{35-55}$ injection as in (A) were either untreated or treated with 4 μ g/ml of MOG $_{35-55}$ for 3 days in the presence of brefeldin A during the last 5 hours of culture. IFN- γ or IL-17-producing CD4 $^{+}$ cells were assayed by intracellular staining followed by flow cytometry. (C) Same as in (B) except that cells were either untreated or treated with MOG $_{35-55}$ or CD3-specific antibody for 3 days. Cell proliferation was measured by one-color cell proliferation kit. (D) $Pias1^{-/-}$ female mice and their wild-type littermates were immunized with MOG $_{35-55}$ and pertussis toxin to induce EAE as described in (17). The development of EAE was scored ($n = 4$). (E) Wild-type and $Pias1^{-/-}$ littermates were immunized as in (D), and lymphocytes were isolated 21 days after the first MOG $_{35-55}$ injection and cultured for 3



days in vitro ($n = 4$). Cytokine production in the cell supernatant was measured by enzyme-linked immunosorbent assay (ELISA). Shown in (A) to (E) is a representative of three independent experiments ($n = 3$ to 7 for each experiment). Error bars represent SEM. P value was determined by unpaired t test.

is a SUMO E3 ligase that binds to chromatin to repress transcription (14, 15). The recruitment of PIAS1 to chromatin requires it to be phosphorylated on Ser 90 . This is induced by a variety of immune regulatory stimuli, including TCR (T cell receptor) activation (16). PIAS1 was phosphorylated on Ser 90 in freshly isolated thymocytes and splenocytes (17) (Fig. 1A). There was a small increase in the percentage of thymic single-positive CD4 $^{+}$ or CD8 $^{+}$ T cells in $Pias1^{-/-}$ mice, although the total T cell number was not significantly altered (fig. S1, A and B). The frequency of both thymic and splenic CD4 $^{+}$ Foxp3 $^{+}$ nT $_{reg}$ cells was significantly increased in $Pias1^{-/-}$ mice (about 80%) (Fig. 1B). In addition, the number of thymic CD4 $^{+}$ Foxp3 $^{+}$ nT $_{reg}$ cells was also significantly increased in $Pias1^{-/-}$ mice (about 135%) (Fig. 1B). However, the mean intensity of Foxp3 expression in Foxp3 $^{+}$ cells was not altered in $Pias1^{-/-}$ cells (fig. S1C). Moreover, $Pias1$ disruption had no significant effect on the in vivo proliferation or survival of T $_{reg}$ cells (fig. S2).

To test whether PIAS1 directly regulates the intrinsic differentiation potential of nT $_{reg}$ cells, bone marrow from wild-type and $Pias1^{-/-}$ mice depleted of CD4 $^{+}$ and CD8 $^{+}$ T cells was transplanted into sublethally irradiated $Rag1^{-/-}$ (recombination-activating gene 1) recipient mice, which lack both T and B cells. $Pias1$ disruption caused a fourfold

increase in the frequency of thymic $CD4^{+}Foxp3^{+}$ cells (Fig. 1C). Transplantation experiments were also performed by injecting $Rag1^{-/-}$ mice with a mixture of bone marrow from wild-type $CD45.1^{+}$ C57SJL mice and either wild-type or $Pias1^{-/-}$ mice ($CD45.2$). The percentage of thymic $CD4^{+}Foxp3^{+}$ from $Rag1^{-/-}$ mice reconstituted with $Pias1^{-/-}$ bone marrow ($CD45.2$) was significantly higher than that of the wild-type controls, whereas no difference was observed in thymic $CD4^{+}Foxp3^{+}$ differentiation of the $CD45.1^{+}$ control T cells (Fig. 1D). These studies suggest that PIAS1 negatively regulates nT_{reg} differentiation.

Peripheral naïve $CD4^{+}$ T cells can be differentiated into so-called induced T_{reg} cells (iT_{reg}) (18–20). In vitro differentiation studies indicate that transient TCR activation positively regulates iT_{reg} differentiation, whereas persistent TCR stimulation antagonizes $Foxp3$ induction (21). $Pias1$ disruption enhanced iT_{reg} differentiation when naïve $CD4^{+}$ T cells were transiently stimulated by TCR alone (~70% increase), but not under the persistent TCR activation conditions (fig. S3A). In addition, $Pias1$ disruption resulted in the inhibition of iT_{reg} differentiation when cells were exposed to a prolonged transforming growth factor- β (TGF- β) treatment under the persistent TCR-stimulating conditions (fig. S3B), possibly

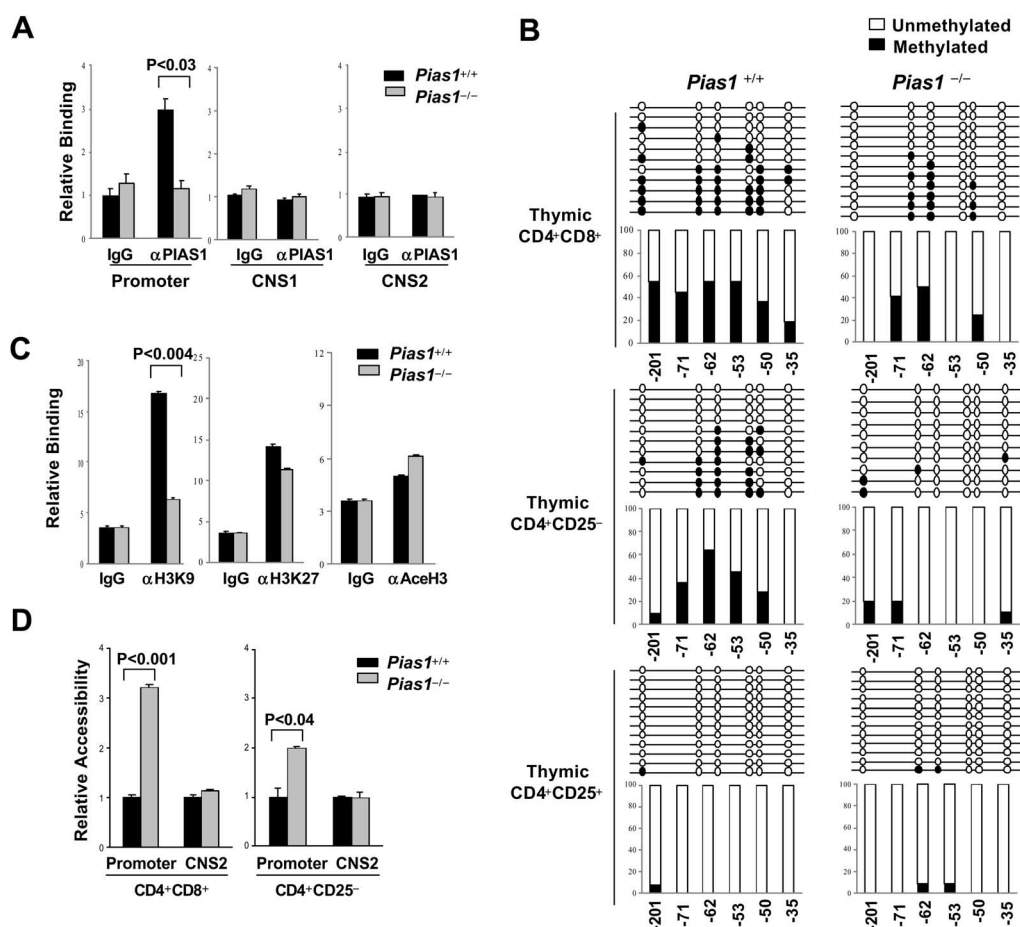
due to the aberrant up-regulation of negative-feedback molecules such as interferon-regulatory factor-1 (22) (fig. S3C).

To test the biological significance of PIAS1-mediated regulation of nT_{reg} in vivo, the response of $Pias1^{-/-}$ mice to myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) was examined. Ten days after MOG immunization, an increased population of $CD4^{+}Foxp3^{+} T_{reg}$ cells in the lymph nodes of $Pias1^{-/-}$ mice was detected, as compared with the wild-type controls (Fig. 2A). The antigen-specific induction of the proinflammatory cytokines interferon (IFN)- γ and interleukin (IL)-17 was significantly suppressed, whereas a higher amount of TGF- β , an immunosuppressive cytokine, was detected in $Pias1^{-/-}$ lymphocytes (fig. S4). Consistently, intracellular staining assays showed a reduced percentage of antigen-specific IFN- γ - or IL-17-producing T cells (Fig. 2B). Furthermore, a defect in lymphocyte proliferation in response to MOG, but not polyclonal CD3-specific antibody stimulation, was observed in $Pias1^{-/-}$ mice (Fig. 2C). The lack of inhibition on $Pias1^{-/-}$ lymphocyte proliferation in response to polyclonal stimulation is likely due to the hyperproliferation of $Pias1^{-/-}$ non- T_{reg} lymphocytes under such conditions (fig. S5). The differentiation of T helper

T_H1 or T_H17 cells was not defective in vitro in the absence of PIAS1 (fig. S6), which suggests that PIAS1 does not have an intrinsic ability to regulate the differentiation of T_H1 or T_H17 cells. $Pias1^{-/-}$ mice displayed resistance toward the development of EAE (Fig. 2D), and significantly reduced amounts of IFN- γ and IL-17, but increased levels of TGF- β , were detected in the lymph nodes of $Pias1^{-/-}$ mice (Fig. 2E). These results are consistent with the notion that the increased T_{reg} cells in $Pias1^{-/-}$ mice contribute to the observed resistance toward the development of EAE.

PIAS1 contains a chromatin-binding domain that targets PIAS1 to gene promoters to regulate transcription (14–16). We tested the hypothesis that $Foxp3$ may be a direct PIAS1-target gene by ChIP (chromatin immunoprecipitation) assays. PIAS1 bound to the $Foxp3$ promoter, but not the two conserved noncoding DNA sequences (CNS1 and CNS2), in thymic $CD4^{+}CD8^{+}$ and $CD4^{+}CD25^{-}$ T cells (Fig. 3A and fig. S7A). In contrast, the binding of PIAS1 to the $Foxp3$ promoter was weak in thymic and peripheral $CD4^{+}CD25^{+}$ cells, consistent with the reduced expression of PIAS1 in these cells (fig. S7B). The $Foxp3$ promoter is hypermethylated in $CD4^{+}CD25^{-}$, but hypomethylated in $CD4^{+}CD25^{+}$ cells (6, 7, 23–25), which inversely correlates with the amounts of PIAS1 expression

Fig. 3. PIAS1 maintains a repressive chromatin state of the $Foxp3$ promoter. (A) ChIP assays were performed with freshly sorted $CD4^{+}CD8^{+}$ thymocytes from male $Pias1^{+/+}$ or $Pias1^{-/-}$ mice ($n = 4$), using PIAS1-specific antibody or IgG. Bound DNA was quantified by quantitative real-time fluorescence polymerase chain reaction (QPCR), with specific primers against the various regions of the $Foxp3$ locus, and normalized with the input DNA. (B) Methylation analysis of the $Foxp3$ promoter was performed by bisulfite conversion of genomic DNA from various sorted T cell populations of wild-type and $Pias1^{-/-}$ male mice ($n = 4$). The x axis represents the positions of the CpG sites relative to the transcription start site (+1) in the $Foxp3$ gene; the y axis represents the percentage. (C) ChIP assays were performed the same way as in (A) except that antibodies against trimethylated H3K9, histone H3 trimethylated at Lys²⁷ (H3K27), or acetylated histone H3 (AceH3) were used, and the primers against the $Foxp3$ promoter region were used. (D) REA assays were performed with thymic $CD4^{+}CD8^{+}$ or splenic $CD4^{+}CD25^{-}$ T cells. The data were quantified by QPCR and expressed as a ratio of digestion at the $Foxp3$ promoter or CNS2 region to digestion at a nondigestible CNS1 region of the $Foxp3$ locus. Shown in (A) to (D) is a representative of three independent experiments ($n = 4$ to 6 for each experiment). Error bars represent SEM. P value was determined by unpaired t test.



(fig. S7B). Bisulfite-sequencing analysis was performed to examine the methylation of the *Foxp3* locus in various subpopulations of wild-type and *Pias1*^{+/+} cells. Several CpG sites in the *Foxp3* promoter were hypermethylated in wild-type thymic CD4⁺CD8⁺, CD4⁺CD25⁺ and splenic CD4⁺CD25⁺ T cells, but were hypomethylated in thymic and splenic CD4⁺CD25⁺ T_{reg} cells (Fig. 3B and fig. S8A). It is noteworthy that *Pias1* disruption caused a significant reduction of DNA methylation of the *Foxp3* promoter (Fig. 3B and fig. S8A). In contrast, the removal of PIAS1 showed no effect on the methylation of the heavily methylated CNS2 element (fig. S8B), which is consistent with the lack of significant PIAS1 binding to the CNS2 region (Fig. 3A). *Pias1* disruption also showed a modest effect on the demethylation of the *Foxp3* promoter in the common lymphoid progenitors and thymic CD4⁺CD8⁺ cells (fig. S9).

To examine whether PIAS1 also plays a role in the methylation of other gene promoters, we performed similar studies on CD25, a key regulator of T_{reg} cell differentiation (4, 6). ChIP studies showed that *Cd25* is a PIAS1-target gene (fig. S10A). *Pias1* disruption resulted in the hypomethylation of the *Cd25* gene promoter in hematopoietic stem

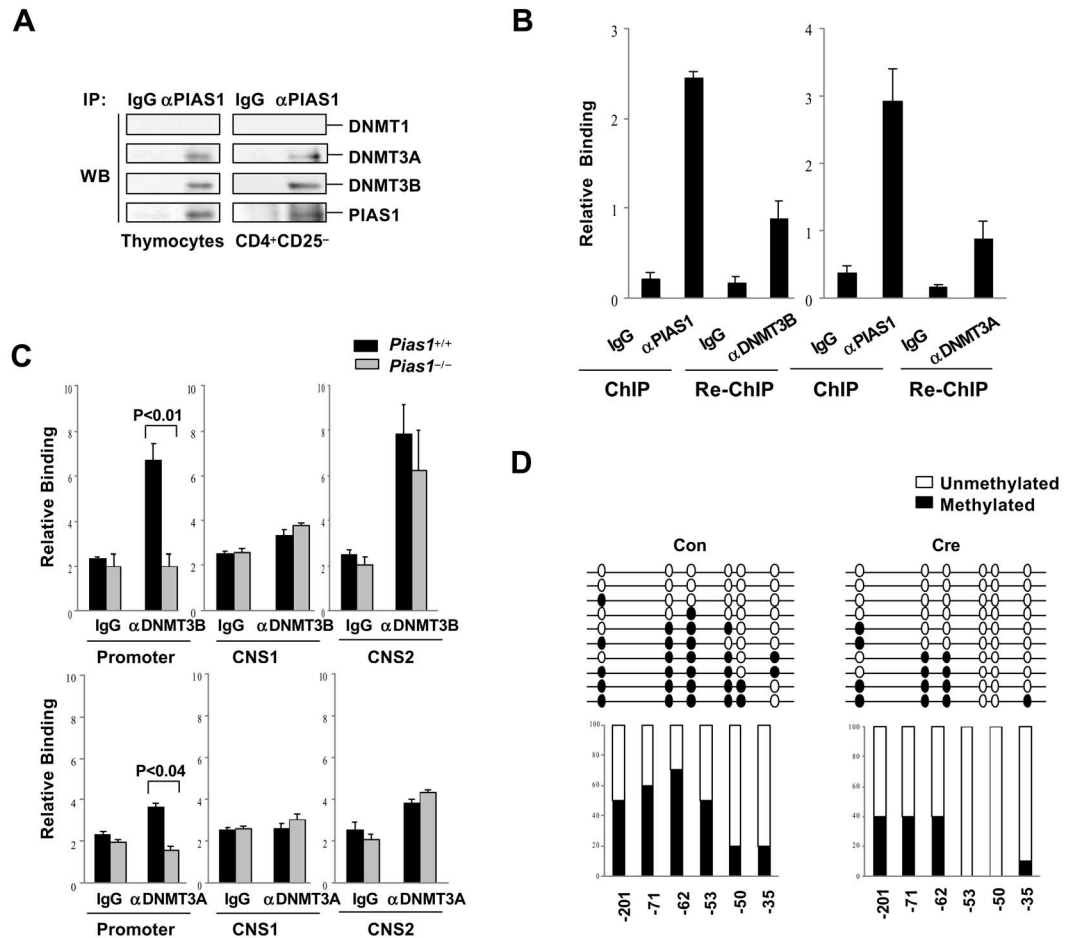
cells and thymic CD4⁺CD8⁺ cells (fig. S10B). Consistently, an increased frequency of CD4⁺CD25⁺ T cells was observed in *Pias1*^{+/+} thymus and spleen (fig. S10C), as well as in transplantation studies (fig. S10, D and E).

We also examined the chromatin status of the *Foxp3* promoter by analyzing histone modifications. ChIP analysis showed a reduction of the repressive histone H3 methylation at Lys⁹ (H3K9) methylation code in *Pias1*^{+/+} CD4⁺CD8⁺ thymocytes (Fig. 3C). In addition, restriction enzyme accessibility analysis (REA) on the *Foxp3* locus (23) showed the enhanced accessibility of the *Foxp3* promoter, but not the PIAS1 nonbinding CNS2 region in *Pias1*^{+/+} thymic CD4⁺CD8⁺ and splenic CD4⁺CD25⁺ T cells (Fig. 3D), which support a more open chromatin structure of the *Foxp3* promoter in *Pias1*^{+/+} T cells. Furthermore, *Pias1* disruption resulted in the enhanced binding of STAT5, a key transcription factor involved in *Foxp3* induction (10–12), to the *Foxp3* promoter (fig. S11A). The amount of STAT5 protein or STAT5 phosphorylation was not affected by *Pias1* disruption (fig. S11, B and C). A significant increase of nuclear factor of activated T cells (NFAT) binding to the *Foxp3* promoter was also observed in *Pias1*^{+/+}

CD4⁺CD8⁺ thymocytes (fig. S11D). Consistently, *Pias1* disruption resulted in the increased frequency of Foxp3⁺ CD4⁺CD8⁺ thymocytes (fig. S12).

We examined whether PIAS1 may maintain a repressive chromatin structure through the recruitment of DNA methyltransferases (DNMTs), which promote DNA methylation of chromatin (26). PIAS1 was shown to interact with DNMT3B in a yeast two-hybrid screen and in 293T cells upon overexpression (27, 28). DNMT3A and DNMT3B, but not DNMT1, were present in PIAS1 immunoprecipitates from thymocytes or splenic CD4⁺CD25⁺ cells (Fig. 4A). Furthermore, sequential ChIP studies indicated that PIAS1 forms a complex with DNMT3A and DNMT3B on the *Foxp3* gene promoter (Fig. 4B). DNMT3B and DNMT3A bound to both the *Foxp3* promoter and the CNS2 region, but not the CNS1 element, in the wild-type CD4⁺CD8⁺ thymocytes (Fig. 4C). *Pias1* deletion completely abolished the binding of both DNMT3A and DNMT3B to the *Foxp3* promoter (Fig. 4C), but not the CNS2 region. The levels of DNMT expression were not affected by *Pias1* disruption (fig. S13). In addition, a modest binding of DNMT1 to the *Foxp3* promoter was observed, which was inhibited by

Fig. 4. PIAS1 is required for the promoter recruitment of DNMT3A and DNMT3B. **(A)** Coimmunoprecipitation assays. Protein extracts from wild-type thymocytes or splenic CD4⁺CD25⁺ T cells were subjected to immunoprecipitation with PIAS1-specific antibody or IgG, followed by immunoblotting with the indicated antibodies. **(B)** ChIP assays were performed with wild-type thymocytes by using PIAS1-specific antibody or IgG. The presence of the *Foxp3* promoter region in the precipitates was quantified by QPCR. In the re-ChIP experiments, PIAS1-specific antibody precipitates were released, reimmunoprecipitated with an antibody against DNMT3A or DNMT3B, and analyzed for the presence of the *Foxp3* promoter sequence. **(C)** ChIP assays were performed with freshly sorted CD4⁺CD8⁺ thymocytes from male *Pias1*^{+/+} or *Pias1*^{+/+} mice (*n* = 4), using an antibody against DNMT3A or DNMT3B. Bound DNA was quantified by QPCR with the specific primers against *Foxp3* promoter, CNS1 or CNS2 region. **(D)** Methylation analysis of the *Foxp3* promoter was performed by bisulfite conversion of genomic DNA from thymocytes of *Rag1*^{+/+} mice reconstituted with either control GFP (Con) or Cre-GFP (Cre) retrovirus-infected *Dnmt3a*^{2lox/2lox}/*Dnmt3b*^{2lox/2lox} bone marrow. The x axis represents the positions of the CpG sites relative to the transcription start site (+1) in the *Foxp3* gene; the y axis represents the percentage. Shown in (A) to (D) is a representative of three independent experiments (*n* = 4 to 6 for each experiment). Error bars represent SEM. *P* value was determined by unpaired *t* test.



Pias1 disruption (fig. S14). The PIAS1-dependent recruitment of DNMTs to the *Foxp3* promoter was also observed in splenic CD4⁺CD25⁺ cells (fig. S15), but not in CD4⁺CD25⁺ T_{reg} cells (fig. S16). Collectively, these results indicate that PIAS1 is associated with DNMT3A and DNMT3B in T cells and is required for their recruitment to the *Foxp3* gene promoter.

To test if DNMT3A and DNMT3B play a role in the *Foxp3* promoter methylation, we performed transplantation experiments using mice in which the functional domains of both *Dnmt3a* and *Dnmt3b* genes are flanked by two loxP sites (*Dnmt3a*^{2lox/2lox}/*Dnmt3b*^{2lox/2lox}) (29, 30). Bone marrow cells from *Dnmt3a*^{2lox/2lox}/*Dnmt3b*^{2lox/2lox} mice were infected with green fluorescent protein (GFP) or Cre-GFP retrovirus, and the deletion of *Dnmt3a* and *Dnmt3b* was confirmed (fig. S17A). Sorted GFP⁺ bone marrow was transplanted into sublethally irradiated *Rag1*^{−/−} mice. Decreased *Foxp3* promoter methylation was observed in thymocytes from the *Rag1*^{−/−} mice reconstituted with the Cre retrovirus-transduced bone marrow (Fig. 4D). ChIP assays indicated that the decreased DNMT3A and DNMT3B expression by the Cre transduction had no substantial effect on the binding of PIAS1 to the *Foxp3* promoter (fig. S17B).

Heterochromatin protein 1 (HP1) plays an important role in promoting H3K9 methylation and is known to interact with DNMTs (31). HP1-γ was strongly associated with the *Foxp3* promoter in wild-type, but not *Pias1*^{−/−}, thymocytes (fig. S18). The PIAS1-mediated epigenetic gene regulation is selective, because the chromatin status of genes such as *Ctla4* was not affected by *Pias1* disruption (fig. S19).

Our studies have identified an epigenetic control mechanism in the negative regulation of Foxp3⁺ nT_{reg} differentiation. PIAS1 acts by maintaining a repressive chromatin state of the *Foxp3*

promoter, at least partly through the recruitment of DNMTs and HP1 to promote epigenetic modifications (fig. S20). *Pias1* disruption results in the formation of a permissive chromatin structure of the *Foxp3* promoter and enhanced promoter accessibility to transcription factors such as STAT5 and NFAT, which lead to the increased probability that precursor cells will differentiate into Foxp3⁺ T_{reg} cells (fig. S20). The physiological role of PIAS1 in the regulation of *Foxp3* gene is supported by the observed increase of the Foxp3⁺ nT_{reg} population in *Pias1*^{−/−} mice and the resistance of *Pias1*^{−/−} mice toward the development of EAE. Thus, the PIAS1 pathway may represent a therapeutic target for the treatment of autoimmune diseases. DNMTs have no intrinsic sequence specificity (26). Our finding that PIAS1 regulates the binding of DNMTs to the *Foxp3* promoter, but not the CNS2 element, suggests that PIAS1 may be an important cofactor that confers specificity in the DNMT-mediated chromatin methylation. The PIAS1-mediated DNA methylation and histone modifications may serve as a fine-tuning mechanism in the control of epigenetic modifications during T cell differentiation.

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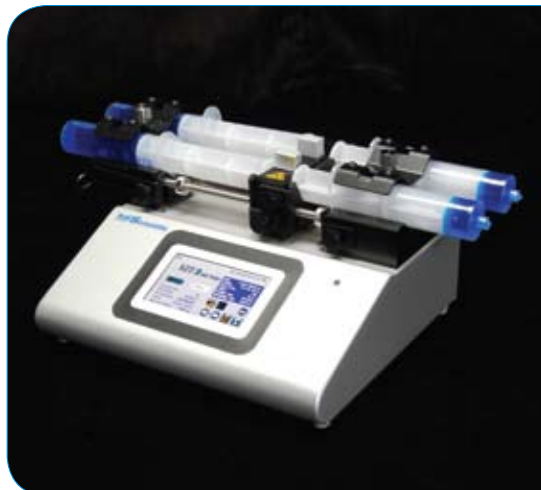
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Supporting Online Material

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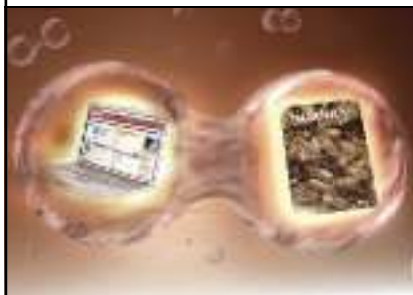
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The University of California, San Diego Health Sciences invites nominations or applications for the position of Chair, Department of Medicine.

The Chair of Medicine, reporting to the Vice Chancellor for Health Sciences, is responsible for the overall management,

academic planning, budget, personnel, resources allocation and program development and implementation for the department. The Chair provides leadership and direction in meeting the clinical, research, educational, and professional missions of the department interfacing with UC San Diego Health System – UC San Diego Medical Center, Hillcrest and Thornton Hospital, La Jolla, the Moores Cancer Center, as well as the VA San Diego Healthcare System and Rady Children's Hospital-San Diego.

Qualifications include a doctoral degree and board certification in any of the medical specialties and a strong track record in clinical medicine and research. Applicants must be board certified in Internal Medicine. Other qualifications are experience in education and research, and significant academic experience and accomplishment, including teaching, research, public service, leadership and contributions to diversity.

The Department of Medicine at UC San Diego is the largest of the School of Medicine departments, with over 420 full-time faculty members, 185 residents and fellows in ACGME-accredited training programs and an annual budget of nearly \$150 million. Its 17 subspecialty divisions include new divisions in Biomedical Informatics and Global Public Health. The department has highly respected residency programs in categorical internal medicine, medicine-pediatrics, and physician-scientist training as well as a preliminary medicine internship, 13 subspecialty fellowship training programs, and a dermatology residency. There is a strong tradition of translational research, cross-disciplinary collaboration, mentorship, clinical excellence and public service in the department. Salary is negotiable and commensurate with qualifications and experience and based on UC pay scales.

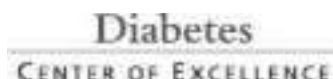
Review of applications will begin **November 30, 2010**, and continue until the position is filled. Submit nominations or letter of application with complete curriculum vitae, bibliography, and names and e-mail addresses of three references to <https://apol-recruit.ucsd.edu/apply>. Please select the following job notice: **MEDICINE Chair, Department of Medicine (10-217) OR by mail to:**

**Department of Medicine Chair Search Committee
c/o Lourdes C. Felix
Office of the Vice Chancellor and Dean
9500 Gilman Drive
La Jolla, California 92093-0602**

UC San Diego is an Equal Opportunity/Affirmative Action Employer with strong institutional commitment to excellence through diversity.



University of
Massachusetts
UMASS Medical School



FACULTY POSITION

The Diabetes Center of Excellence at the University of Massachusetts Medical School invites applications for a **SENIOR TENURED or JUNIOR TENURE-TRACK** basic science or clinical investigator faculty position. The Diabetes Center of Excellence currently consists of basic and physician scientists representing a broad range of disciplines in the biomedical and clinical sciences, with members from several Medical School Departments and Programs working together as central New England's leading center for diabetes clinical care, innovation, and discovery. The Center occupies state-of-the-art research space on an expanding Medical School campus, and benefits from being a continuously funded Diabetes-Endocrinology Research Center (DERC) for over 28 years. Basic investigators benefit from core facilities for deep sequencing, proteomics, genotyping, fluorescence-activated cell sorting, digital imaging/confocal microscopy, genomics/bioinformatics, transgenic/knockout mice, and mouse metabolic phenotyping. Clinical and community investigators benefit from infrastructure for recruitment and retention of research participants, measurement, behavioral and nutritional intervention resources and facilities, and academic-community research partnerships. Adult and pediatric clinical efforts of the Diabetes Center are housed in a new Ambulatory Clinical Care building designed so that patients can receive appropriate retinal photos, foot care, diabetes education, and other routine medical care at one location. The position will be highly competitive with regard to start-up funds, laboratory space and salary. The Center seeks an individual of outstanding research potential relating to diabetes pathophysiology or treatment.

Applicants should send curriculum vitae, statement of research interests, and names and addresses of three references to:

**Dr. David M. Harlan
Co-Director, Diabetes
Center of Excellence
Search Committee Chair
% Patricia Cannon
55 Lake Avenue North
Ambulatory Care Building,
Room AC4.127
Worcester, MA 01655
Patricia.cannon@umassmed.edu
508.856.3800**

As an Equal Opportunity and Affirmative Action Employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives, and backgrounds.

Formed in 1986 and comprising 25 European member states, EUMETSAT's role is to establish, operate and exploit European meteorological satellite systems. Data from these systems are essential for precise and accurate weather forecasting; they also assist global earth observation and climatological programmes and directly benefit national economies by enabling marine, agricultural, aviation and other industries to plan and act more effectively.

Currently the systems include two generations of geostationary Meteosat satellites whose global overview is now complemented by the detailed observations provided by polar orbiting MetOp satellites and Jason-2.

Optical Scientist for Sentinel-3 Satellites

(Ref. 10/23)

In this role, you will support the advancement of the understanding and utilisation of optical instruments on low earth orbit satellites, in particular those embarked on the Sentinel-3 satellites. You will also be responsible for scientific developments to improve the use of data from optical instruments on Sentinel-3. To be successful, you should have a University degree in Remote sensing, Oceanography, Meteorology, Physics or equivalent. A strong background in the scientific and technical aspects of remote sensing, with special expertise in infrared optical instruments is also needed.

Training Officer

(Ref. 10/21)

In this role, you will contribute to the enhancement of data use from EUMETSAT's satellite programmes in EUMETSAT Member States and Co-operating States and in developing countries. You will coordinate and organise training activities in oceanography, land surface use and atmospheric composition to widen the user base of EUMETSAT satellite data. To be successful, you should have a University degree in remote sensing, meteorology or oceanography, as well as a background in the application of satellite observing systems in operational meteorology, oceanography or related disciplines.

Based in Darmstadt, Germany, the posts are offered on an initial four-year contract. We offer an attractive remuneration package including a highly competitive salary, private social security and health coverage, generous allowances, as well as financial support and practical assistance in relocating your family (where applicable).

EUMETSAT is committed to providing an equal opportunities work environment for men and women. Please note that only applications from nationals of one of the EUMETSAT Member States can be considered.

Further information on the positions, salary and benefits, and how to apply can be found on the EUMETSAT web site: www.eumetsat.int.

Closing date: 14th November (Ref: 10/23) and 21 November 2010 (Ref: 10/21).

Member States: Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Luxembourg, The Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom





TEXAS TECH UNIVERSITY

Edward. E. Whitacre Jr.
College of Engineering

The Maddox Chairs in Energy at Texas Tech University

The Edward E. Whitacre Jr. College of Engineering at Texas Tech University is committed to leveraging these **two exceptionally large endowed chairs at over \$7 million each**, to become one of the nation's leaders in finding solutions to the world's energy challenges. The college is seeking world-class researchers in solar and sustainable energy as candidates for the Maddox Chairs.

Donovan Maddox Distinguished Engineering Chair in Solar Energy Jack Maddox Distinguished Engineering Chair in Sustainable Energy

Candidates are expected to have an international reputation in the fields of solar energy or energy sciences and engineering as evidenced by publications, citations, and peer recognition. In addition, a record of acquiring external resources to support research, team building, and mentoring of associates and graduate and undergraduate students is necessary.

The successful candidates will set the tone, vision, and path in order to build an internationally recognized program at Texas Tech University in solar and sustainable energy research. The appointments will be as a full professor in the Whitacre College of Engineering.

The holders of each of the Maddox Chairs will be expected to not only bring his or her own research activities to the Whitacre College of Engineering, but also to build a collaborative community of scholars at Texas Tech to build a world-class research program.

Screening will begin upon the receipt of applications and will continue until the position is filled. Candidate names will not be made public until the final stages of the search.

Curriculum vitae and the names and contact information of at least four references should be submitted at www.coe.ttu.edu/maddox. To nominate a colleague for these chairs, visit www.coe.ttu.edu/maddox. Nominations can be made anonymously.

Questions about the Jack Maddox or Donovan Maddox Chairs should be directed to:

Jack Maddox and Donovan Maddox Search Committees
Texas Tech University | Whitacre College of Engineering
Box 43103 | Lubbock, Texas 79409-3013 | engineeringdean.coe@ttu.edu | 1.800.528.5583

Texas Tech University | Whitacre College of Engineering
1.800.528.5583 | www.coe.ttu.edu/maddox

An Affirmative Action/Equal Opportunity/
Americans with Disabilities Employer

OKLAHOMA

Case-Hooper Professorship of Zoology: Neurobiology

We seek an outstanding researcher in **Neurobiology**, for appointment at the level of **tenured Associate Professor** or **tenure-track Assistant Professor**, as the inaugural holder of the endowed **Case-Hooper Professorship of Zoology** at the **University of Oklahoma**, beginning in fall 2011. This professorship offers a unique opportunity to combine research and teaching in neurobiology with a leading role in an active interdepartmental Cellular & Behavioral Neurobiology Graduate Program (www.ou.edu/cbn). The successful candidate will have a Ph.D. degree and a demonstrated ability to conduct significant independent research as evidenced by publications. The candidate will be expected to establish and maintain a distinguished, externally funded research program and contribute to undergraduate and graduate teaching in the Department of Zoology. Applicants should submit a cover letter, complete curriculum vitae, selected reprints/preprints, research and teaching statements, and names and contact information for three references, as PDF files to **Chair, Case-Hooper Professorship Search Committee**, at casehooper@ou.edu. Further information is available at <http://zoology.ou.edu>. Screening of candidates will begin **December 15, 2010**, and will continue until the position is filled.

Department of Zoology, 730 Van Vleet Oval, Room 314, University of Oklahoma, Norman, OK 73019, USA

The University of Oklahoma is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are encouraged to apply.



Worcester Polytechnic Institute Department of Chemistry and Biochemistry Department Head

WPI invites applications and nominations for Head of the Department of Chemistry and Biochemistry. The Department is supported by a new, state-of-the-art facility at Gateway Park, housing the Life Sciences Bioengineering and Chemistry research facilities which opened in the spring of 2007 along with a robust community of faculty and students. WPI seeks a Department Head with a clear and creative vision for building and sustaining ambitious departmental research programs while maintaining a focus on excellence in teaching and has the skills necessary for the day to day management of the department activities and administrative staff. The Chemistry and Biochemistry Department Head is responsible for coordinating with the other Heads across the wider WPI community the development of interdisciplinary research teams for the purpose of securing external funding. Our vision for the new Head includes enhancing the department's role in the natural sciences at all levels and providing strategic positioning for new faculty appointments as we grow. The department offers BS, MS and Ph.D. degrees.

To learn more about research at WPI and the Chemistry and Biochemistry Department visit www.wpi.edu/research and www.wpi.edu/Academics/Depts/Chemistry

The Department Head will have an earned doctorate from one of the fields of chemistry or biochemistry, an international reputation in research, and a distinguished record of publication and funding. She or he must demonstrate outstanding leadership and mentoring abilities as well as a commitment to high quality teaching at both the undergraduate and graduate levels.

The application should consist of a detailed curriculum vita, a letter of intent that describes professional interest (research, teaching, and administrative), and names of a minimum of three references. Applications and nominations should be sent to cbcheads@wpi.edu. Further inquiries can be directed to the Office of the Dean of Arts & Sciences, 508-831-4678 or email dofcarcik@wpi.edu.

U.S. News and World Report consistently ranks WPI among the top national universities and recently placed WPI in its top 30 for faculty resources. Further information about WPI and the department can be accessed at www.wpi.edu

To enrich education through diversity,
WPI is an affirmative action, equal opportunity employer.
— A member of the Colleges of Worcester Consortium. —

GRADUATE PROGRAM



The Leipzig School of Human Origins - An International Max Planck Research School -

by

The University of Leipzig
and

The Max Planck Institute for Evolutionary Anthropology

The **Leipzig School of Human Origins** offers a unique interdisciplinary graduate program to study the evolutionary history of humans and great apes. Graduate students are accepted into one of the following areas, but are encouraged to take part in courses and seminars from all three disciplines:

Comparative and Molecular Primatology

Evolutionary and Functional Genomics, Ancient DNA, Molecular Anthropology and Genome Bioinformatics

Human Paleontology, Prehistoric Archaeology and Archaeological Science

The language of the school is English. Visit www.leipzig.de for information on living in Leipzig, Germany, in the center of Europe.

For project and application details go to www.leipzig-school.eva.mpg.de or contact us at:

e-mail: leipzig-school@eva.mpg.de

phone: ++49 (0) 341 3550-0

fax ++49 (0) 341 3550-119

Application deadline: January 31, 2011



APPOINTMENT OF DIRECTOR, INSTITUTE FOR ANIMAL HEALTH

Pirbright, Surrey, UK



You are a global leader in your field. Now is your chance to lead the UK Institute for Animal Health into the new decade.

The UK Government and the Biotechnology and Biological Sciences Research Council have committed over £130m to deliver a state-of-the-art high biocontainment laboratory complex at Pirbright. Construction is underway and the Institute is poised to enter an exciting future supported by world-class facilities and a new scientific strategy, which focuses on viral diseases in livestock. The IAH will also have a key role as part of the multi-disciplinary Food Security global grand challenge.

The BBSRC is looking for an inspirational leader to be the next Director of the IAH who will lead the Institute through a period of significant growth.

You will be a scientist with an established international reputation in research in virology or other disciplines relevant to IAH's new scientific focus and will be responsible for promoting and positioning the Institute as a world-leading facility. You will maximise opportunities for the development of strategic partnerships and collaborations with a range of stakeholders; provide input into policy making decisions; offer advice and guidance on matters to do with viral diseases in livestock; work with industry on the development of diagnostics and vaccines; and collaborate with universities in the training of new generations of scientists and academic vets.

As Director of the IAH you will be responsible for all aspects of the Institute's business including the definition and implementation of its new scientific strategy and maintaining its long term sustainability and development. The IAH is already recognised by its World Reference Laboratory status for diseases such as Foot and Mouth and Morbillivirus.

It is expected that you will wish to maintain active research interests and thus financial and facilities support will be provided. Applicants with a strong background in the 'One Health' agenda or medical virology would also be considered.

An attractive salary plus relocation assistance and a performance related non-consolidated element is available commensurate with the level and strategic importance of the post.

Further information, including instructions on how to apply and/or an informal confidential telephone discussion, may be obtained from BBSRC's recruitment consultants, Carbon-NfP. Please contact Sharon Taylor by email sharon.taylor@carbon-nfp.com in the first instance.

The closing date for applications is Friday, 12 November 2010.

Carbon-NfP Ltd, 17 Russell Court, Woburn Place, London WC1H 0LL.

BBSRC welcomes applications from all sections of the community. As part of the disability symbol we guarantee to interview all disabled applicants who meet the minimum criteria for the role.



www.carbon-nfp.com



Yale

Faculty Positions
Department of Cell Biology
Yale University School of Medicine

Yale University - Biodesign Institute

Yale University, to further the development of its West Campus research enterprise, is seeking faculty at both junior and senior ranks for the new Biodesign Institute, which is being founded to broadly develop the interface between quantitative cell biology and bio-inspired design on the nanoscale. The Institute is directed by James E. Rothman, Chair of Cell Biology and Professor of Chemistry, and its deputy director is Kyle Vanderlick, Dean of the School of Engineering and Applied Science and Professor of Chemical Engineering, and will be located in dedicated space on the West Campus. Faculty in this Institute will have a primary appointment in the life science or physical science departments in the Faculty of Arts and Sciences, the School of Engineering and Applied Science, or the School of Medicine. Candidates must have a Ph.D. or M.D. degree in a relevant discipline and a record of research that demonstrates originality and productivity in addressing significant questions. Relevant research areas include, but are not limited to membrane biochemistry and biophysics, cellular and bio-inspired nanomachines, and active materials such as DNA-based objects, and dynamic optical microscopy on the nanoscale.

To apply, please submit to Biodesign.search@yale.edu as PDF files with the subject heading "Biodesign Search" the following materials: your complete CV with a list of publications, a summary of doctoral research [1 page], a summary of postdoctoral research [max. 2 pages], and a research plan [max. 3 pages], and up to five reprints of published work, and arrange for three letters of recommendation addressed to **The Biodesign Search Committee, c/o Patricia Sullivan** and e-mailed to the above address. The review of applications will begin on **November 8, 2010**.

Yale University is an Affirmative Action, Equal Opportunity Employer. Yale values diversity among its faculty, students, and staff and strongly encourages applications from women and underrepresented minorities.



Faculty positions in Bioinformatics, Computational Biology, Genomics and Systems Biology

Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China

Beijing Institute of Genomics (BIG) invites applications for 3-5 faculty positions in **Bioinformatics, Computational Biology, Genomics and Systems Biology**. Candidates should hold a Ph.D. degree and, in order to qualify for the "100 talent" award from CAS, should have 4 years of training after the Ph.D degree. Areas of interest include bioinformatics, computational biology, large-scale epigenetic studies, genomics and closely related disciplines. Demonstrated experience in large-scale genomic data acquisition and analysis is highly desirable. BIG has state-of-the-art genomic platforms for high throughput sequencing, genotyping, and high-performance computing. The new faculty members will join an interactive, interdisciplinary community of scientists at CAS and move in our brand new facilities located in Beijing Olympic Village. BIG offers a competitive startup package, including substantial research funds, supporting staff, and family relocation benefits.

Candidates should e-mail their application, preferably in PDF format, to job@big.ac.cn. The application should include a curriculum vitae, a description of past research, a description of proposed research (3-7 pages), and copies of three representative publications. Candidates should arrange to have three signed letters of reference in PDF format sent by e-mail to job@big.ac.cn.

CHAIR, DEPARTMENT OF NEUROLOGICAL SURGERY

UMDNJ-New Jersey Medical School is seeking a qualified applicant to serve as Chair of the Department of Neurological Surgery. The Chair will offer leadership and vision for the continued growth of the research, clinical and educational missions of the department as well as oversee departmental administrative, financial and program components.

Candidates for the position must be eligible for appointment to the academic rank of Professor and have a demonstrated record of outstanding leadership and accomplishment. International recognition in clinical, research, and educational endeavors is expected with strong experience in mentoring faculty.

The Department of Neurological Surgery has become a highly recognized clinical and academic department and is considered among the top neurological surgery programs in the country. Clinically, the department performs approximately 2400 procedures per year, maintains a fully accredited seven year neurosurgical residency training program, and has consistently seen an increase in case volume annually. Critical collaborations for the department include the Departments of Neurology and Neurosciences, Psychiatry, Medicine, Radiology, Ophthalmology & Visual Science, Surgery and Physical Medicine & Rehabilitation.

UMDNJ is the largest health sciences university in the nation and NJMS anchors its largest campus in the city of Newark. The campus provides easy access to east coast cities including New York, Philadelphia, and Washington, DC to offer the benefits of both the metropolitan area and the nearby quiet suburbs with their highly ranked school systems.

Interested individuals should send a curriculum vitae and cover letter to **Kenneth Maiese, MD, Chair of the Neurological Surgery Search Committee**, c/o Michael Petti, Executive Assistant to the Dean, UMDNJ-New Jersey Medical School, 185 South Orange Avenue, MSB C-671, Newark, NJ 07101-1709 via email (pettime@umdnj.edu) or standard mail.



**NEW JERSEY
MEDICAL SCHOOL**

University of Medicine & Dentistry of New Jersey



TEMASEK RESEARCH FELLOWSHIP (TRF) 2010

The Nanyang Technological University (NTU) and the National University of Singapore (NUS) invite outstanding young researchers with a PhD Degree in science or technology to apply for the prestigious TRF awards.

The TRF scheme provides selected young researchers an opportunity to conduct and lead research that is relevant to defence. It offers:

- Three year research grant, with an option to extend up to a further three years,
- possible tenure-track academic appointment with the university at the end of the TRF,
- attractive and competitive remuneration.

Fellows may lead and conduct research, and publish in these areas:

1. Biomimicry
2. Cognitive Sciences
3. Cyber Security
4. Computational Photography
5. Microsystem Technologies

Other fundamental areas of science or technology, where a breakthrough would be of interest to defence and security, will also be considered.

Singapore is a globally connected cosmopolitan city-state with a supportive environment and vibrant research culture. For more information and application procedure, please visit:

NTU – <http://www3.ntu.edu.sg/trf/>

NUS – <http://www.nus.edu.sg/dpr/funding/trf.htm>

Closing date: 11 January 2011

Shortlisted candidates will be invited to Singapore to present their research plans, meet local researchers and identify potential collaborators in April/ May 2011.



Office of the Science and
Technology Adviser to the
Secretary of State

Jefferson Science Fellowship

The National Academies is pleased to announce a call for applications for the 2011 Jefferson Science Fellows program. This program is a model for engaging the American academic science, technology and engineering communities in the formulation and implementation of U.S. foreign policy.

Jefferson Science Fellows spend one year at the U.S. Department of State or the U.S. Agency for International Development in Washington, DC and may periodically travel to U.S. foreign embassies and/or missions.

Jefferson Science Fellow awards are open to tenured academic scientists, technologists and engineers from U.S. institutions of higher learning. Applicants must be U.S. citizens and will be required to obtain a security clearance. Detailed information on the Jefferson Science Fellows program is available online:

www.nas.edu/jsf

The deadline for applications for the 2011 program year is
January 14, 2011.

The Jefferson Science Fellows program is sponsored by the U.S. Department of State and U.S. Agency for International Development.

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine



KOCH INSTITUTE
for Integrative Cancer Research at MIT

Koch Institute Clinical Investigator: Physician Scientist

The David H. Koch Institute for Integrative Cancer Research at MIT is seeking applicants for newly created Koch Institute Clinical Investigator positions. These three-year extendable fellowship positions will be filled by physician-scientists interested in bridging the gap between basic science and clinical cancer care through conducting basic and translational oncology research at the Koch Institute concurrent with maintaining clinical activity at one of the area oncology treatment centers. Candidates must be subspecialty board-certified and be eligible for licensure by the Massachusetts Board of Medical Examiners. Interested individuals from postdoctoral, instructor and assistant professor levels are all encouraged to apply. A record of productivity in clinical, translational, and basic research is essential. The selected individuals will work closely with faculty mentors at the new Koch Institute, an NCI designated cancer center (web.mit.edu/ki/), to build a state-of-the-art basic/translational research program with dedicated lab space and research support. Applicants should include curriculum vitae, a brief description of research interests, and request two letters of recommendation be sent under separate cover.

Interested candidates should apply online at
<http://academicjobsonline.org> by January 15, 2011

Additional inquiries may be directed to:
Executive Director's Office, David H. Koch Institute for Integrative Cancer Research at MIT, 77 Massachusetts Avenue, E18-601, Cambridge, MA 02139, Attn: Mary Heckbert
Phone: (617) 324-2169, Fax: (617) 324-2044
Email: ki-jobs@mit.edu

The David H. Koch Institute for Integrative Cancer Research at MIT is an Equal Employment Opportunity/Affirmative Action Employer.



**Massachusetts
Institute of
Technology**

UNIVERSITY PROFESSOR POSITIONS

The Pohang University of Science and Technology (POSTECH) is Korea's first research-oriented university and is known for its unique contribution to the advancement of science and technology in Korea. POSTECH is ranked first among Asia's specialized universities in the 2010 Chosun Ilbo - QS Asian University Rankings.

POSTECH invites distinguished scholars to join in POSTECH's efforts to become a global leader in science & technology research.

Position Announcement:

We are seeking distinguished scholars in all fields of science and engineering. Appointments will be made for multiple positions at the rank of University Professor. POSTECH will provide startup funds of up to 7 million US dollars for each distinguished scholar. Salary will be competitive and commensurate with candidate's qualifications and experience. The exact amount of financial support may vary depending on the field.

Desired Qualifications:

- Nobel laureates, Fields medalists or scholars of equivalent caliber
- Members of world-renowned national academies of sciences/engineering such as the National Academy of Sciences (USA), the National Academy of Engineering (USA), and the Royal Society (UK)
- Editors of the world's top scientific journals or those with equivalent experience
- Those who have demonstrated potential to grow to become a University Professor are also welcome to apply.

Interested individuals should send a CV, a statement of research plans, and a teaching statement to **e-mail: recruit@postech.ac.kr** or **mailing address: Faculty Search Committee, Office of Academic Affairs, POSTECH, San 31, Hyoja-dong, Nam-gu, Pohang 790-784, Republic of Korea.**



Faculty Positions in Cardiovascular & Metabolic Disorders

We invite applicants with a PhD, MD or equivalent, and a record of outstanding promise and achievements, for our faculty positions in cardiovascular and metabolic disorders. The Duke-NUS Cardiovascular and Metabolic Disorders (CVMD) Signature Research Program will bring together top-flight researchers investigating the interactions between metabolic disorders and cardiovascular disease, focusing on translational discoveries that can impact clinical care. Investigators will address diverse aspects of the problem from diabetes and dyslipidemia to hypertension and vascular disease.

Positions include full salary as well as generous startup and 5 years of annual research funding to assure a stable base of support to be supplemented by competitive awards. Candidates should submit a cover letter, curriculum vitae, summary of research accomplishment and an outline of future plans by **February 1, 2011** to:

Thomas Coffman, MD, Director
Cardiovascular and Metabolic Disorders Program
Duke-NUS Graduate Medical School Singapore
8 College Road, Singapore, 169857
E-mail: cvmd.recruit@duke-nus.edu.sg

ABOUT DUKE-NATIONAL UNIVERSITY OF SINGAPORE GRADUATE MEDICAL SCHOOL

Duke-NUS brings post-baccalaureate, research-intensive medical education and research to Asia, and represents a truly global partnership between Duke University in the U.S. and National University of Singapore (NUS). Duke-NUS shares a modern campus with Singapore's largest hospital, Singapore General Hospital, and several national centers, including the National Heart Centre. The facilities in nearby Biopolis also provide a unique array of complementary resources. In addition, the CVMD program has close associations with the Duke Cardiovascular Research Center and Duke Global Health Institute.

www.duke-nus.edu.sg



Assistant/Associate Professor of Biosciences and Deputy Director of the State Virology Lab

The University of Alaska Fairbanks, Institute of Arctic Biology and the Alaska State Virology Lab seek a medical virologist with combined administrative and academic roles. This successful candidate will: (1) plan, organize, and direct the activities of the State Public Health Virology Laboratory located in Fairbanks, Alaska (Deputy Director), and (2) conduct academic research and teaching for the University of Alaska Fairbanks as part of a growing statewide biomedical program. In addition to the administrative component of the Deputy Director position, approximately half of the effort will be dedicated to a traditional faculty role with an emphasis on research. A successful candidate will also be an Alaska IDeA Network of Biomedical Research Excellence (INBRE) (<http://www.alaska.edu/inbre/>) investigator, and is expected to contribute to the INBRE infectious disease research focus area. The Alaska INBRE program offers an attractive research startup package and other forms of support for research and outreach. Duties will include but are not limited to: Administrative functions of overall budgetary and program planning for the State Virology Laboratory (SVL) and the candidate's research program, establishing goals for the SVL, providing consultation in clinical and medical virology, preparing in-depth reports, position papers, research studies and testimony before special or regular legislative committees, and providing consultation visits to local hospital, clinical and private laboratories, and other appropriate agencies. As Deputy Director of a High Complexity Testing Laboratory refer to the requirements set forth in 42 CFR 493.1445(e), and as a Clinical Consultant, High Complexity Testing Laboratory (Virology) refer to the requirements set forth in 42 CFR 493.1457. The successful candidate is expected to collaborate with University of Alaska researchers and is expected to participate in curriculum development for the biomedical sciences.

Applications must be submitted online at <https://www.uakjobs.com>, posting number **0060566** or quicklink: www.uakjobs.com/applicants/Central?quickFind=71691. If you need assistance with the application process, please contact the UAF Human Resource Department at (907) 474-7700.

*The University of Alaska is an Equal Employment/Affirmative Action
Employer and Educational Institution.*



Endowed Professorship in Cancer Therapeutics

The Department of Medicinal Chemistry and Molecular Pharmacology in the College of Pharmacy and the Purdue University Center for Cancer Research invite applications from individuals with a distinguished record of accomplishment to fill an endowed chair in Cancer Therapeutics at the rank of Full Professor. We are particularly interested in candidates who apply techniques and approaches emerging from the chemistry-biology interface or nanoscience to the discovery, development, and/or delivery of cancer therapies.

The Department offers a unique multidisciplinary environment in which chemistry and biology are applied to the improvement of human health, with particular strengths in cancer drug discovery. The Purdue University Center for Cancer Research is one of only seven NCI-designated Cancer Centers nationwide focused exclusively on basic and translational research. It maintains strong programs in drug delivery and molecular sensing, medicinal chemistry, chemical and structural biology, and cell growth and differentiation. Discovery Park at Purdue University offers a unique environment for collaborative interdisciplinary research. The Bindley Bioscience and Birck Nanotechnology Centers and the newly founded Purdue Drug Discovery Institute offer opportunities for collaborations on drug targets and the application of nanotechnology to therapeutics. The NIH-funded Indiana Clinical and Translational Sciences Institute provides a nexus for translational research in therapeutics, bringing together basic and clinical research efforts from Purdue, Notre Dame, and Indiana University. More information on this position can be found at: <http://www.mcmp.purdue.edu/aboutus/facultypositions.php>.

Applicants should send curriculum vitae, a brief summary of research accomplishments and of ongoing research, to: **Head of Cancer Therapeutics Search, Purdue University, MCMP, 575 Stadium Mall Dr, West Lafayette, IN 47907 and/or electronic submission to Angie Schutz, e-mail: arschutz@purdue.edu**. Applications will be reviewed beginning **October 15, 2010**, and will continue until the position is filled.

Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer fully committed to achieving a diverse workforce.



FACULTY POSITION IN COMPUTATIONAL MATERIALS SCIENCE AND ENGINEERING AT DREXEL UNIVERSITY

The Department of Materials Science and Engineering at Drexel University (www.mse.drexel.edu) is seeking applications for a tenure/tenure-track faculty position with a demonstrated record of excellence in computational materials science and materials engineering. This position will be offered at a rank commensurate with the qualifications of the applicant. While primary consideration will be given to candidates with areas of expertise in materials for energy conversion and storage, the ideal applicant should possess research interests that are synergistic with one or more existing strengths of the department, including nanomaterials, polymers, electronic materials, biomaterials, complex oxides, materials in extreme environments and advanced microstructural design and processing. Additional consideration will be given to applicants who have the capacity to introduce computational methods and simulation to students as a tool for guiding student learning of principles of materials science and engineering at both the undergraduate and graduate levels. Outstanding students, an accomplished faculty, dedicated staff, and investments in infrastructure and in key instrumentation within a staffed materials characterization facility in recent years have contributed to enhancing the quality of our academic programs and the visibility of our research profile, which was recently ranked #11 among all materials PhD programs in the US by the National Research Council.

Applicants should submit a cover letter; a full curriculum vitae; statements of research and teaching plans; and three letters of recommendation online at <http://www.materials.drexel.edu/faculty/positions/>. Applications received by **December 31, 2010**, will receive full consideration.

Drexel University is an Equal Opportunity Employer and encourages applications from qualified women and minorities.



University of California, Berkeley Departments of Bioengineering, Chemical and Biomolecular Engineering and the Energy Biosciences Institute (EBI)

TENURED or TENURE-TRACK FACULTY POSITION IN MICROBIAL, BIOCHEMICAL AND METABOLIC ENGINEERING, SYSTEMS AND SYNTHETIC BIOLOGY

The University of California at Berkeley seeks applicants at both the senior and junior levels for a tenured/tenure-track faculty position in the general area of microbial bioengineering with an expected start date of July 1, 2011. Of particular interest are individuals whose research includes metabolic engineering and/or systems and synthetic biology; however, creative and energetic individuals who show extraordinary promise or accomplishment in related areas will also be considered.

Applicants must have a Ph.D. and evidence of outstanding scholarship within a relevant discipline. We encourage applications from candidates with the communication skills and cross-cultural abilities to maximize effective collaboration with a diverse community of campus and external colleagues.

This position is sponsored by the Energy Biosciences Institute, which will provide significant resources and collaborative opportunities for individuals to develop a leading-edge program in biofuels research (<http://www.energybiosciencesinstitute.org>). Both startup and multi-year research funds will be available from the EBI for the bioenergy components of the individuals' research programs. Candidates hired into these positions would also be free to seek support for research in other areas.

Applications should be submitted online at <http://bioeng.berkeley.edu/career/ebifaculty.php> and should include a resume, statements of research and teaching interests, selected publications, and the names of three references. While online application submission is preferred, we will also accept application materials sent by mail to the **Department of Bioengineering, 306 Stanley Hall, UC Berkeley, Berkeley, CA 94720-1762**. Applications received through **March 1, 2011**, will be considered; however the review of applications will commence on **November 15, 2010**. Candidates will be reviewed on an ongoing basis, and early application is recommended.

*The University of California is an
Equal Opportunity, Affirmative Action Employer.*



FACULTY POSITIONS IN BIOENGINEERING

The Department of Bioengineering in the College of Engineering at the University of California, Berkeley invites applications for two tenure-track or tenured positions at the assistant, associate, or full professor level in bioengineering, with an expected start date of July 1, 2011.

The Berkeley Department of Bioengineering enjoys a close relationship with the School of Medicine at the University of California, San Francisco (UCSF). Our interdisciplinary program offers outstanding opportunities for collaboration with distinguished researchers in related departments and colleges at Berkeley, UCSF, the Lawrence Berkeley National Laboratory, and within the greater Bay Area biotech community. This exceptional environment for teaching and research in a rapidly growing field will provide the successful candidate with a unique opportunity to provide intellectual and technological leadership in bioengineering.

We seek two individuals with demonstrated excellence in the broad areas of translational and cellular engineering, including but not limited to regenerative medicine and drug delivery. Applicants must have a Ph.D. and evidence of outstanding scholarship within a relevant discipline. Successful applicants will be expected to teach undergraduate and graduate courses in bioengineering and should have a strong commitment to and potential for excellence in teaching and leadership. To learn more about our department please visit <http://bioeng.berkeley.edu>. The level of appointment will be based on experience and qualifications.

Applications should be submitted online at <http://bioeng.berkeley.edu/career/bioefaculty.php> and should include a resume, statements of research and teaching interests, selected publications, and the names of three references. While online application submission is preferred, we will also accept application materials sent by mail to the **Department of Bioengineering, 306 Stanley Hall, UC Berkeley, Berkeley, CA 94720-1762**. Applications received through **March 1, 2011**, will be considered; however the review of applications will commence on **November 15, 2010**. Candidates will be reviewed on an ongoing basis, and early application is recommended.

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Equal Opportunity, Affirmative Action Employer.*



Visitor Program

Collaborative Research Opportunities in Neutron Science

- Visiting Student Thesis Research Program
- Visiting Post-doctoral Research Program
- Faculty Research Sabbaticals

The Neutron Scattering Science Division at the Oak Ridge National Laboratory (ORNL) announces new collaborative opportunities for visiting researchers. Possibilities exist for faculty sabbaticals during the summer or academic year. In addition, it is anticipated that funding will be available for short- and long-term research placements for university-based postdoctoral fellows and graduate students to pursue research using neutron scattering at ORNL. This will allow students, post-doctoral fellows, and their academic mentors to participate in exciting world-leading research in collaboration with ORNL scientific staff, using unique state-of-the-art facilities at the Spallation Neutron Source and High Flux Isotope Reactor. Additional benefits include the chance to meet and interact with many other leading scientists who visit ORNL's neutron scattering facilities to pursue their own research. The scientific areas are open to any branch of materials-based research that can use neutron scattering, including Biology, Chemistry, Engineering, and Physics.

Applications and visiting start dates will be considered on an ongoing basis, but for full consideration for visits during the summer of 2011, applications are due by December 31, 2010.

Additional details can be found at: <http://neutrons.ornl.gov/crv> or contact us at nsvisit@ornl.gov

Industrial Liaison

The Oak Ridge National Laboratory (ORNL) invites applications for a Neutron Sciences Industrial Liaison in the Neutron Sciences Directorate. With the United States' highest-flux, reactor-based neutron source for condensed matter research (the High Flux Isotope Reactor) and the world's most intense pulsed, accelerator-based neutron source (the Spallation Neutron Source), ORNL is becoming the world's foremost center for neutron science. Research at these facilities encompasses the physical, chemical, materials, biological, and medical sciences and will provide opportunities for up to 2000 researchers each year from industry, research facilities, and universities all over the world.

The Neutron Sciences and the Technology Partnerships Directorates jointly seek candidates with outstanding records of accomplishment and demonstrated ability to build world-leading innovative programs of neutron scattering research in partnership with industrial organizations. Candidates with research experience in relevant scientific disciplines are invited to apply. The liaison is expected to build comprehensive programs that increase the awareness and use of neutron scattering techniques in applied research programs in collaboration with industrial partners. Such programs are expected to maximize the impact of ORNL research capabilities in the applied sector and link to similar capabilities in other ORNL directorates, government agencies, and associated universities.

For more information about this position and to apply, visit jobs.ornl.gov.

neutrons.ornl.gov

Assistant Professor Institute for Immunology



Penn Medicine

The Institute for Immunology at the University of Pennsylvania School of Medicine seeks candidates for an Assistant Professor position in the tenure track. Under the directorship of Dr. Steve Reiner, the Institute for Immunology was formed to promote novel immunologic strategies for preventing and treating infectious and immune-mediated diseases. Individuals working in any area of immunology are welcome to apply but preference will be given to those who utilize innovative, state-of-the-art experimental and computational approaches to understand basic and/or translational problems of relevance to human immunology. The environment for collaborative research within the Institute and across Departments, Schools, and Centers at The University of Pennsylvania is outstanding. The successful applicant must have a clear record of productivity and ingenuity during their post-doctoral training and high likelihood of securing extramural funding. The faculty appointment will be in an appropriate department in the School of Medicine.

A generous recruitment package, as well as excellent institutional and core research resources will be provided. Applications will not be considered after November 15, 2010.

The University of Pennsylvania is an equal opportunity, affirmative action employer. Women and minority candidates are strongly encouraged to apply.

Apply for this position online only at:
http://www.med.upenn.edu/apps/faculty_ad/index.php/g323/d2420



Texas State University-San Marcos is a member of The Texas State University System.
Department of Biology

The Department of Biology (<http://www.bio.txstate.edu>) at Texas State University (<http://www.txstate.edu>) invites applications for two positions. The successful candidates will be expected to develop externally funded research programs involving students and teach both graduate and undergraduate courses in the Department of Biology. Salary and startup package are negotiable. A letter of application, CV, and the names and contact information of five people willing to serve as references should be sent, as a single PDF, to the addresses given below. Review of applications will begin on December 15 and continue until the positions are filled.

Cell Biology – The position is tenure-track at the rank of assistant professor. To be considered, an applicant must have a Ph.D. in the life sciences or related field and a record of research accomplishments in eukaryotic cell biology. Preference will be given to applicants with postdoctoral experience in cell biology, a demonstrated ability to develop an externally funded research program, expertise in confocal and electron microscopy, expertise at the interface of molecular biology and biochemistry, expertise that complements existing departmental strengths, and the ability to conduct research within existing University infrastructure. Inquiries may be directed to **Dr. Dana García** (DG08@txstate.edu). Applications should be sent to cellbio@txstate.edu.

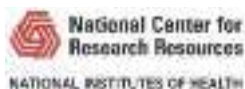
Wildlife Management and Conservation – Rank and tenure status are negotiable. To be considered, an applicant must have a Ph.D. in wildlife science or related field, and a peer-reviewed publication record in wildlife management and/or conservation. Preference will be given to applicants with postdoctoral experience, a record of procuring extramural funding to support a student-centered research program, a demonstrated ability to collaborate with wildlife and conservation agencies and non-governmental agencies, a record of successfully mentoring students, a demonstrated ability to teach wildlife management courses, and expertise in using quantitative tools to conduct field research to address regional and national wildlife management and conservation issues that are a result of human-driven changes in habitats and landscapes. Inquiries may be directed to **Dr. Butch Weckerly** (FW11@txstate.edu). Applications should be sent to wildlife-management@txstate.edu.

Texas State University is a large (~33,000 students) doctoral-granting university located on the eastern edge of the Texas Hill Country between Austin and San Antonio.

Texas State University-San Marcos will not discriminate against any person (or exclude any person from participating in or receiving the benefits of any of its activities or programs) on any basis prohibited by law, including race, color, age, national origin, religion, sex or disability, or on the basis of sexual orientation.

Positions @ NIH

THE NATIONAL INSTITUTES OF HEALTH



**Director, Division of Biomedical Technology
National Center for Research Resources
National Institutes of Health
Department of Health and Human Services**



THE POSITION: The National Center for Research Resources (NCRR) is seeking exceptional candidates for the position of Director, Division of Biomedical Technology. This individual will lead a program that supports research to discover, create, and develop innovative technologies and provides access to these technologies to the biomedical research community. This research is conducted through grants for technology-driven research and development, the biomedical informatics research network, and biomedical technology research centers. The incumbent will supervise a staff of eleven, including nine scientific professionals in overseeing 50 Biomedical Technology Research Center Grants in five broad technology areas: Imaging, Informatics, Optical and Laser Technology, Technology for Structural Biology and Technology for Systems Biology. At these research centers, interdisciplinary teams create unique, transformative technologies and work to promote their widespread use. These innovations are accomplished through a synergistic interaction of technical and biomedical expertise, both within the centers and through intensive collaborations with other leading laboratories. These research centers span basic, translational and clinical research to create tools that advance science at the molecular level and change scientific approaches to diagnosis and treatment of disease. Additionally, the Division administers the shared instrumentation program which provides institutions with the funds to purchase state of the art technologies, helping researchers remain at the forefront of the biology and medicine. Lastly, the Division supports biomedical researchers through investigator-initiated research projects or large collaborative research projects with other NIH organizations and/or Federal agencies.

The NCRR provides laboratory scientists and clinical researchers with the environments and tools they need to understand, detect, treat, and prevent a wide range of diseases. www.ncrr.nih.gov This support enables discoveries that begin at a molecular and cellular level, move to animal-based studies, and then are translated to patient-oriented clinical research, resulting in cures and treatments for both common and rare diseases. This position offers a unique and exciting opportunity for an extremely capable individual to share responsibility in providing strong and visionary leadership to an organization dedicated to enhancing our understanding of health and disease, translating basic research into medical care, and improving human health.

QUALIFICATIONS REQUIRED: Applicants must possess a Ph.D., or equivalent degree, as well as senior-level research experience or knowledge of research programs moving research from the basic laboratory sciences into clinical research. Candidates should be outstanding communicators and known and respected as distinguished individuals of outstanding competence. Applicants should also demonstrate the ability to think strategically, work collaboratively, and use a consultative approach to problem solving and decision making.

SALARY/BENEFITS/OTHER INFORMATION: Salary is commensurate with experience and a full package of Civil Service benefits is available, including: retirement, health and life insurance, long term care insurance, leave and savings plan (401K equivalent). The National Institutes of Health inspires public confidence in science by maintaining high ethical principles. In addition to the Federal government's code of ethics, we have our own agency specific standards which are described at the NIH Ethics web site. <http://ethics.od.nih.gov/overview.htm> This position is subject to a background investigation.

HOW TO APPLY: A Curriculum Vitae, Bibliography, and two letters of recommendation must be received by **November 16, 2010**. Application packages should be sent to the **National Institutes of Health, National Center for Research Resources, ATTN: Sabrina Posley, 6701 Democracy Boulevard, Suite 206, Bethesda, Maryland 20892** or e-mail at sposley@mail.nih.gov.

For further information, please call **(301) 435-0717**. All information provided by candidates will remain confidential and will not be released outside the NCRR search process without a signed release from candidates.



WWW.NIH.GOV

Tenure/Tenure Track Investigator Position Laboratory of Immunology

The Laboratory of Immunology (LI), Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, invites applications for a tenure/tenure-track investigator position in immunology. Applicants should have a Ph.D., M.D., or equivalent degree; an outstanding record of postdoctoral accomplishment; and an interest in any area of biomedical research related to immunology.

Specifically, we seek a highly creative individual who will establish an independent, world-class research program that takes full advantage of the special opportunities afforded by the stable, long-term funding of the intramural research program at NIH. Applicants should be interested in developing and applying novel approaches to the study of problems of major biological and/or medical importance, which could include a significant clinical or translational effort in addition to bench research. The successful candidate would have access to the NIH Clinical Center, a state-of-the-art research hospital on the NIH campus in Bethesda, MD, and would have ample opportunity to participate in the activities of the Center for Human Immunology and other trans-NIH initiatives involving technology development, translational investigation, and multidisciplinary science.

Generous ongoing support for salary, technical personnel, postdoctoral fellows, equipment, and research supplies will be provided. Available cores or collaborative facilities include flow cytometry, advanced optical imaging, microarray generation and analysis, high throughput sequencing, computational biology, production of transgenic and gene-manipulated mice, biosafety level (BSL)-3 facilities, chemical genomics, and support for projects involving RNAi screening. In addition to an outstanding, international

postdoctoral community, a superior pool of graduate and undergraduate students is available to the successful applicant.

NIAID's Laboratory of Immunology has a distinguished history of accomplishment in immunology. We strongly encourage application by outstanding investigators who can continue and enhance this record of achievement. Current LI investigators are Ronald Germain, Michael Lenardo, David Margulies, Stefan Muljo, William Paul, Ethan Shevach, and Tsan Xiao.

To apply, e-mail your curriculum vitae, bibliography, and an outline of a proposed research program (no more than two pages) in PDF format to Ms. Bao-Hanh Ngo at LIT-TTSearch@niaid.nih.gov. In addition, three letters of reference must be sent directly from the referee to Drs. Giorgio Trinchieri and Dan Kastner, Co-Chairs, NIAID Search Committee, c/o Ms. Bao-Hanh Ngo at LIT-TTSearch@niaid.nih.gov or 10 Center Drive, MSC 1356, Building 10, Room 4A22, Bethesda, MD 20892-1356. E-mail is preferred.

Applications will be reviewed starting **December 13, 2010**, and will be accepted until the position is filled. For further information about this position, contact Dr. William Paul at 301-496-5046 or wpaul@niaid.nih.gov.

A full package of benefits (including retirement and health, life, and long-term care insurance) is available. Women and minorities are especially encouraged to apply. U.S. citizenship is not required.

Further information on working at NIAID is available on our Web site at www.niaid.nih.gov/careers/LIS.

National Institute of Allergy and Infectious Diseases



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health



National Institute of Allergy and Infectious Diseases

Proud to be Equal Opportunity Employers



University of California, Irvine School of Biological Sciences Assistant Professor Positions Available

The School of Biological Sciences at the University of California, Irvine invites applications for 4 new tenure-track positions at the rank of Assistant Professor. We seek candidates with Ph.D. and/or M.D. degrees who have demonstrated excellence, originality and productivity in research, and are ready to establish a vigorous and internationally competitive research program.

Department of Developmental and Cell Biology invites applications in all areas of developmental biology, and its interface with other disciplines.

Department of Ecology and Evolutionary Biology invites applications for a tenure-track position in Comparative and Evolutionary Animal Physiology. We seek applicants who use any combination of theoretical and/or experimental approaches to address major issues in animal physiology. We are particularly interested in individuals whose research spans multiple levels of biological organization in the context of evolution, ecology and/or behavior.

Department of Molecular Biology and Biochemistry wishes to recruit in the area of Gene Expression. Research areas of interest might include, but are not limited to: genome organization, post-translational control, viral gene regulation, and molecular control over host-pathogen interactions.

Department of Neurobiology and Behavior engages in interdisciplinary approaches to the study of neurobiology, with an emphasis on neural plasticity and behavior. Current research themes include mechanisms of: learning and memory, neurodegenerative disorders, sensory neuroscience, neural development and the neurobiology of substance abuse. Preference will be given to applicants whose research integrates with, or complements, one or more of these themes.

The successful applicants are expected to conduct strong research programs, to contribute to the teaching of undergraduate and graduate students, and to complement existing strengths in each Department. Start-up funds will be provided to establish a competitive research program, and salary will be based upon the University of California pay scale. Interested applicants should submit through the specified on-line recruitment system: (1) cover letter; (2) curriculum vitae; (3) summary of research accomplishments and goals; (4) a statement of teaching philosophy; and, (5) contact information for 3 references. To receive full consideration, material should be received by **December 1, 2010**. URL: http://jobs.bio.uci.edu/showopenjobs_tenure.cfm for application instructions under each department.

The University of California, Irvine is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants, including women and minorities.



Washington University in St. Louis SCHOOL OF MEDICINE

Outstanding candidates are invited to apply for tenure-track positions at the assistant and/or associate professor level as part of a newly established multidisciplinary Center for the Study of Itch. Candidates are expected to develop independent and innovative research programs focusing on itch. This initiative has been developed by the Department of Anesthesiology and the Division of Dermatology, Department of Medicine, to promote interdisciplinary studies of mechanisms of itch and to translate these findings into treatments for patients suffering from intractable itch. Candidates with strong interests in the study of itch are encouraged to apply. Research areas for this recruitment include but are not limited to: cutaneous biology, electrophysiology, GPCRs/channel signal transduction, immunology and imaging.

Applicants should submit a CV, a statement of research experience and future plans, and have three reference letters sent to: chenz@wustl.edu.



Tenure Track Faculty Position in Bacteriology Department of Microbiology University of Washington

The Department of Microbiology at the University of Washington in Seattle is conducting a search for an Assistant Professor in the fields of environmental microbiology, bacterial diversity, physiology, and/or prokaryotic cell biology. We are looking for an innovative investigator who will develop an independent research program studying bacterial physiological, metabolic and structural diversity; bacterial responses to environmental signals and stress factors; microbial genetics and evolutionary processes; or fundamental microbial processes. The position is a 12-month, full-time, tenure-track position in the School of Medicine. In addition to research, the new faculty member will support the department's teaching mission, including teaching at the undergraduate or graduate level. All University of Washington Faculty engage in teaching, research and service.

Salary and benefits are competitive and will be commensurate with the qualifications and experience of the applicant. Applicants with a Ph.D., 1-2 years postdoctoral experience and a strong publication record should send their CV, a one or two page statement of research interests and the names and contact information for three references to: **Chair, Bacteriologist Search Committee, Department of Microbiology, Box 357242, Room K357B, University of Washington, 1705 N.E. Pacific Street, Seattle WA 98195**. Application materials may also be sent by e-mail c/o **Bonnie Hightower** at [bbh@uw.edu](mailto:bhb@uw.edu). Deadline for receipt of applications is **December 1, 2010**.

The University of Washington is an Affirmative Action, Equal Opportunity Employer and has built a culturally diverse faculty. Applications from female and minority candidates are especially encouraged. Expanding opportunities for women and other underrepresented groups in the faculty is an important goal of the department.



Download your free copy today at
ScienceCareers.org/booklets

Positions @ NIH

THE NATIONAL INSTITUTES OF HEALTH



Deputy Director

National Human Genome Research Institute

The National Human Genome Research Institute (NHGRI), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), seeks to identify an outstanding Deputy Director.

The NHGRI Deputy Director will assist the Director in providing overall leadership of the Institute, sharing responsibilities in all phases of leading the preeminent organization dedicated to advancing genomic and genetic research, including its clinical applications. As a member of the NHGRI senior leadership, the Deputy Director will work with the Director in shaping and executing a strategic vision for the Institute as well as communicating that vision to the Institute staff and the broader scientific community. In working closely with the Director, the Deputy Director helps to develop Institute goals, priorities, policies, and program activities; this requires staying abreast of developments and needs of the Institute and the field.

Applicants must have an M.D. and/or Ph.D or equivalent degree in the biomedical sciences, as well as a broad knowledge of the field of human genetics and genomics. They must further have a compelling vision for the future of the field and the role for NHGRI within the field. Also required are senior-level research and/or clinical experience and knowledge of the major scientific areas related to genetics and genomics, in addition to well-honed administrative and interpersonal skills to meet the demands of helping to lead a complex organization. Applicants should have demonstrated leadership in dealing with different stakeholder groups within the research community, planning and assessing programs, developing plans to resolve operational problems and issues, and managing financial and human resources. Applicants should be known and respected within their profession, both nationally and internationally as individuals of outstanding scientific competence.

Salary is competitive and will be commensurate with the candidate's experience. A full Federal benefit package is available, including retirement, health and life insurance, long-term care insurance, annual and sick leave, and the Thrift Savings Plan (401K equivalent).

Interested applicants should submit a cover letter that includes a brief description of research, clinical, and/or administrative experience, a current curriculum vitae and bibliography, names and contact information of five references, and a brief written vision for becoming the NHGRI Deputy Director. Questions about the position and applications themselves should be sent to Ms. Ellen Rolfes via email at ellenr@exchange.nih.gov. All information provided by the candidates will remain confidential and will not be released outside the NHGRI search process without a signed release from the candidate.

Applications will be reviewed starting November 1, 2010, and will be accepted until the position is filled.

DHHS and NIH are Equal Opportunity Employers and encourage applications from women and minorities.

NATIONAL HUMAN GENOME RESEARCH INSTITUTE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov



TENURE TRACK POSITION IN IMMUNOLOGY

The Experimental Immunology Branch (EIB), Center for Cancer Research (CCR) of the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Bethesda, MD, invites applications for a tenure track principal investigator position in basic molecular or cellular immunology. The applicant should have a Ph.D., M.D. or equivalent degree, a strong record of scientific accomplishments, and the potential to establish an independent research program in any aspect of molecular or cellular immunology. The position provides salary and full funding to establish an independent research program, including laboratory space, equipment, budget, technical personnel, and support for fellows. Salary will be commensurate with education and experience. The EIB consists of 8 Principal Investigators: Alfred Singer, Richard Hodes, Andre Nussenzweig, Hyun Park, Paul Roche, David Segal, Stephen Shaw, and Dinah Singer. Active research covers a wide range of areas of immunology including: thymic education and T cell differentiation, cytokine receptor signaling, genetic recombination and chromosomal instability, inflammation biology, antigen presentation, receptor assembly and transport, signal transduction, and regulation of gene expression. Scientific interactions are encouraged and occur extensively among members of EIB as well as with other scientists at the NIH. Applicants should send a CV and bibliography, outline of a proposed research program (no more than two pages), and three letters of recommendation to **Caroline McCabe** at ncieibsearch@mail.nih.gov. Applications must be received by **November 15, 2010**.



Post-doctoral Fellow Functional genomics of T cell malignancies

The National Institutes of Health (NIH) in Bethesda, Maryland, the world's largest medical research facility, seeks applications from exceptional candidates for the position of Post-doctoral Fellow in the Waldmann laboratory of the Metabolism Branch, Center for Cancer Research, National Cancer Institute. This individual will collaborate with the Staudt laboratory of the Metabolism Branch to utilize genomic technologies to discover essential genes in T cell lymphoma/leukemia. This 2-year renewable position in the National Cancer Institute is an outstanding opportunity for a scientist who wishes to study the molecular pathogenesis of cancer in a stimulating and well-supported environment. The Fellow will use both RNA interference genetic screens and high-throughput RNA resequencing (RNA-Seq) to uncover essential genes in T cell malignancies. The "Achilles heel" RNA interference genetic screens can identify genes that are required for the proliferation and survival of cancer cells (Ngo et al. Nature 2006 441:106; Shaffer et al. Nature 2008 454:226; Bidere et al. Nature 2009 458:92). The essential pathways revealed by these screens lead to the discovery of oncogenic activating mutations in cancer biopsies, which can be discovered comprehensively by RNA-Seq (Lenz et al. Science 2008 319:1676; Davis et al. Nature 2010 463:88). The Fellow will investigate the aberrant regulatory networks in T cell lymphoma/leukemia revealed by these genomic approaches and devise therapeutic strategies to capitalize on these insights. The successful candidate will have an M.D. and/or Ph.D. degree and a strong publication record in molecular biology, immunology, cancer biology or related fields. United States citizenship is not required but proficiency in English is essential. Please submit curriculum vitae and 3 letters of reference to:

Thomas A. Waldmann, M.D., Chief, Metabolism Branch, CCR, NCI, National Institutes of Health, 9000 Rockville Pike, Bldg. 10, Rm. 4N115, Bethesda, MD 20892.



Nontraditional Careers: Opportunities Away From the Bench Webinar

Want to learn more about exciting and rewarding careers outside of academic/industrial research? View a roundtable discussion that looks at the various career options open to scientists across different sectors and strategies you can use to pursue a nonresearch career.

**Now Available
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Participating Experts:

Dr. Lori Conlan

*Director of Postdoc Services,
Office of Intramural Training and Education
National Institutes of Health*

Pearl Freier

*President
Cambridge BioPartners*

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Science Careers

From the journal *Science*

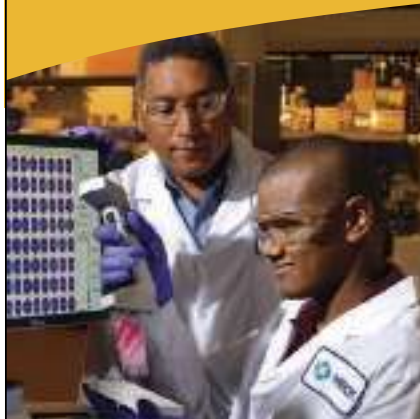


FELLOWSHIPS

Science Scholarships and Fellowships

UNCF/MERCK SCIENCE INITIATIVE

The UNCF/Merck Science Initiative is an innovative approach that creates opportunities in the biological, chemical and engineering sciences for African American students throughout the country.



UNDERGRADUATE

Science Research Scholarship Awards

- Scholarships up to \$25,000
- A paid summer internship at a Merck facility with stipend totaling more than \$5,000
- Mentoring and networking opportunities
- Eligibility: College juniors, science or engineering majors, 3.3 GPA

GRADUATE

Science Research Dissertation Fellowships

- Fellowships up to \$53,500
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree candidates engaged in dissertation research in the biological, chemical or engineering fields

POSTDOCTORAL

Science Research Fellowships

- Fellowships up to \$92,000
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree recipients in the biological or chemical research fields

APPLY ON-LINE

<http://umsi.uncf.org>
Submit by December 1, 2010

T 703 205 3400

F 703 205 3550

E uncfmerck@uncf.org



GENERAL ELIGIBILITY REQUIREMENTS:

Must be African American and a U.S. citizen or a permanent resident



University of Nebraska-Lincoln
Institute of Agriculture and Natural Resources
Position Announcement



DEAN AND DIRECTOR, AGRICULTURAL RESEARCH DIVISION (ARD) INSTITUTE OF AGRICULTURE AND NATURAL RESOURCES (IANR) UNIVERSITY OF NEBRASKA – LINCOLN (UNL)

The Institute of Agriculture and Natural Resources (IANR) at the University of Nebraska-Lincoln (UNL) is seeking a dynamic leader to serve as the Dean of the Agricultural Research Division and Director of the Nebraska Agricultural Experiment Station. The Dean and Director will have the unprecedented opportunity to shape the University's life and social sciences research agendas by capitalizing on campus momentum to grow IANR's and UNL's research enterprise. Campus and system-wide initiatives (e.g. the development of Nebraska Innovation Campus which will emphasize water, food and energy; \$50 million Global Water for Food system-wide initiative; the campus-wide Life Sciences initiative and a \$1.2 billion Foundation campaign) offer multiple opportunities for the dean to leverage agriculture and natural resources research resources (e.g. 40,000+ acres, 200+ research faculty FTE, 4 research centers across the state, and an annual budget of \$80 million including \$40 million in grants and contracts for FY 2009) to serve the research needs of the state, nation and world. The dean will have the opportunity for high visibility and high leadership impact. Frequent interaction by the dean with other administrative leaders on campus, across the country, as well as stakeholders, including commodity organizations, will offer opportunities for innovation and risk-taking that will shape the future of agriculture and natural resources research. This position is available immediately. Additional information on the position and the Agricultural Research Division can be found by visiting the web site: <http://ard.unl.edu>.

Qualifications: The successful candidate shall possess the following qualifications and characteristics: a doctorate or comparable terminal degree; rank of full professor or qualifications for rank of full professor; and a demonstrated record of significant and successful administrative and academic achievement at a research university, or equivalent experience in government or industry.

A demonstrated record of the following qualifications and characteristics are preferable in a candidate: effectiveness as a research leader with administrative experience addressing issues beyond the departmental level; promoting and enhancing diversity and human rights; working with stakeholders and fostering community partnerships; making and implementing difficult decisions in a timely, collaborative, and coordinated manner; successful management of research budgets and personnel matters; providing leadership in working with faculty, administrators, staff, and students in a manner that exemplifies high integrity, openness, accountability, and shared governance; excellence and innovation; collaboration among disciplines as well as integration of basic and applied research; and experience at a land-grant institution.

Salary is competitive and commensurate with qualifications and experience. The University of Nebraska offers a benefits package that makes available group life, health, disability insurance and family coverage programs to the employee; TIAA/CREF and/or Fidelity Investment fund retirement plans; excellent vacation and sick leave plans; and staff and dependent tuition remission.

Nominations: Submit nominations to the Search Advisory Committee Chair at the following address:

Dr. Susan Fritz, Search Advisory Committee Chair
c/o Office of the NU Vice President and IANR Vice Chancellor
University of Nebraska-Lincoln
P. O. Box 830708, 202 Agricultural Hall
Lincoln, NE 68583-0708

Applications: Parties interested in making application should access the web site: <http://employment.unl.edu>, search for requisition number 100601 and complete the faculty academic administrative information form. Attach a letter of application, curriculum vitae, and contact information (mailing address, phone number, and e-mail address, if available) for three professional references. Review of applications will begin **December 1, 2010** and continue until the position is filled or the search is closed.

The University of Nebraska has an active National Science Foundation ADVANCE gender equity program, and is committed to a pluralistic campus community through affirmative action, equal opportunity, work-life balance, and dual careers.

POSITIONS OPEN



THE UNIVERSITY OF PENNSYLVANIA Department of Physics and Astronomy Tenure-Track Assistant Professor Position

The Department of Physics and Astronomy at the University of Pennsylvania (Penn) invites applications for a tenure-track assistant professor appointment in the general area of experimental biological physics. We are particularly interested in scientists who will pursue research programs that complement and enhance Penn's university-wide nanoscience and nanotechnology initiative. In addition to joining departmental efforts in cell signaling, single-molecule studies, molecular self-assembly, and biosensors, the successful candidate will enjoy the benefits of numerous interdisciplinary centers including the Laboratory for Research on the Structure of Matter, the Institute for Medicine and Engineering, the Penn Muscle Institute, and the Nano-Bio Interface Center.

Applications should be submitted online at **website: <http://facultysearches.provost.upenn.edu/applicants/Central?quickFind=50808>** and include curriculum vitae, research statement, and the contact information for three individuals who will provide a letter of recommendation. Review of applications will begin December 15, 2010, and will continue until the position is filled.

The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer. Applications from women and minority candidates are strongly encouraged.

BIOMOLECULAR SCIENCES

East Tennessee State University Department of Biological Sciences invites applications for a tenure-track **ASSISTANT PROFESSOR** beginning August 15, 2011. Ph.D. required in Biology or related areas, with postdoctoral experience preferred. Primary teaching duties are upper level biochemistry with additional molecular courses at undergraduate and graduate level, and mentoring of undergraduate and graduate student research. Applicants must show potential for research excellence and develop an extramurally funded research program in an area of biomolecular science, biochemistry, or molecular biology. We encourage applicants who will enhance diversity and serve as a role model for a diverse student population. Apply to the position at **website: <https://jobs.etsu.edu/applicants/Central?quickFind=51268>**. Questions may be directed to **Dr. Dhirendra Kumar** at e-mail: **biochemist@etsu.edu**. For more information, refer to **website: <http://www.etsu.edu/biology>**.

Application review will begin on December 1, 2010. *ETSU is an Affirmative Action/Equal Opportunity Employer. We encourage applications from or information about women and minority candidates.*

POSTDOCTORAL FELLOWSHIPS

The Geophysical Laboratory, Carnegie Institution of Washington, invites applications for postdoctoral fellowships. The Geophysical Laboratory emphasizes interdisciplinary experimental and theoretical research in fields spanning geoscience, microbiology, chemistry, and physics. The Laboratory supports world-class facilities in high-pressure research; organic, stable isotope and biogeochemistry; mineral physics and petrology; and astrophysics.

Please see **website: http://www.gl.ciw.edu/employment/Postdoctoral_Positions** for information on the application process. Also, see **website: <http://www.gl.ciw.edu/>** for a listing of personnel, current research interests, major facilities, and application information.

Completed applications for Carnegie fellowships should be submitted by December 31, 2010, to: **Russell J. Hemley, Director, Attn: Danielle Appleby, Geophysical Laboratory, 5251 Broad Branch Road, NW, Washington, DC 20015-1305, USA; e-mail: dappleby@ciw.edu**, is preferred.

The Geophysical Laboratory is an Equal Opportunity Employer.

POSITIONS OPEN



MOLECULAR PHYSIOLOGIST

The Department of Biological Sciences at the University of Wisconsin-Milwaukee seeks applicants for a tenure-track position in molecular physiology at the rank of **ASSISTANT PROFESSOR**. We seek candidates with research interests in the cellular and molecular mechanisms by which signaling pathways in the endocrine or nervous systems are disrupted by environmental perturbations. Candidate qualifications:

Minimum qualifications includes a Doctorate degree in Molecular Biology, Physiology, Toxicology, or related field; and postdoctoral research and teaching experience in the areas of physiology/toxicology and/or eukaryotic molecular biology.

Preferred qualifications include demonstrated ability to establish an independent, extramurally funded research program involving M.S. and Ph.D. students.

To apply, please go to **website: <http://www.jobs.uwm.edu/applicants/Central?quickFind=5075>**. A completed application should include: cover letter, curriculum vitae, statement of research goals, statement of teaching interests, and letters of professional reference. Applicants should arrange to have three letters of reference sent as PDF attachments to the departmental chair (e-mail: **daads@uwm.edu**) or mailed to: **Molecular Physiologist Search, Attn: Dr. Saffarini-Chair, Department of Biological Sciences, University of Wisconsin-Milwaukee, PO Box 413, Milwaukee, WI 53201**. Screening of candidates will begin November 15, 2010, and continue until the position is filled. Appointment begins August 2011.

UWM is an Affirmative Action/Equal Opportunity Employer.

The University of Alabama at Birmingham, Department of Vision Sciences

A position at the **ASSISTANT or ASSOCIATE PROFESSOR** level at the University of Alabama at Birmingham (UAB) is available. This will be a tenure earning or tenured position depending upon the candidate's experience. The ideal candidate for this position will have a successful research program with a strong record of extramural support including currently active funding. Candidates with expertise in central visual processing and/or ocular motor systems in appropriate animal models are particularly encouraged to apply.

UAB is one of America's premier research universities, ranking among the top 25 in funding from the National Institutes of Health. Vision research is especially strong at UAB with 54 faculty members from eleven departments constituting the NEI P30-funded Vision Science Research Center. The successful candidate will have access to newly renovated laboratory space that is configured for studies in awake, behaving animals. MRI facilities include two 3T human systems, a 9.4T small animal system, and a vertical 4.7T system dedicated to non-human primate research. He/she will also have access to fully staffed core resources which include electronics, computer, machine shop, histology, molecular biology, transgenic, hybridoma, confocal microscopy, fluorescence resonance energy transfer (FRET), and multiphoton imaging. Vision Sciences faculty actively participate in many graduate programs including Vision Science, Graduate Biomedical Sciences, and Biomedical Engineering.

Applicants should send their curriculum vitae, the names of three references, and a statement of research interests to:

**Dr. Kent T. Keyser, Ph.D.
Department of Vision Sciences
University of Alabama at Birmingham
924 South 18th Street
Birmingham, AL 35294-4390**

Applications should be received no later than December 31, 2010.

The University of Alabama at Birmingham is an Equal Opportunity/Affirmative Action Employer with a strong commitment to ethnic and cultural diversity among its faculty, staff, students, and administrative staff. Applications from women and ethnic minorities are encouraged.

POSITIONS OPEN

WESTERN MICHIGAN UNIVERSITY Assistant Professor in Microbiology Assistant Professor in Plant Biology

The Department of Biological Sciences at Western Michigan University (WMU) seeks applications for two tenure-track **FACULTY POSITIONS**, one in Microbiology and another in Plant Biology, beginning in fall 2011 pending budgetary approval. Preference will be given to applicants who use integrative techniques to investigate fundamental aspects of biology and who have the potential to interact with faculty in the department.

Successful candidates will be expected to participate in the teaching of undergraduate and graduate students, establish extramurally funded research programs, and serve on Departmental, College, and University committees. Modern laboratory facilities as well as generous startup packages for qualified applicants can be anticipated. Information concerning the Biological Science Department's programs and faculty can be obtained at **website: <http://www.wmich.edu/bios/>**. A Ph.D. and relevant postdoctoral experience is required. Western Michigan University is a student-centered research institution and U. S. News and World Report top 100 public university, with a growing graduate program that offers a unique opportunity for individuals seeking a balanced research and teaching career. The Carnegie Foundation for the Advancement of Teaching has placed WMU among the 76 public institutions in the nation designated as research universities with high research activity.

Applicants should visit **website: <http://www.wmich.edu/hr/careers-at-wmu.html>** to apply (**posting #0601344** for Microbiologist and **#0601342** for Plant Biologist). For more information contact **John Spitsbergen, Chair, Department of Biological Sciences (e-mail: john.spitsbergen@wmich.edu)**. Review of applications will begin on November 15, 2010, and continue until the positions are filled. *WMU is an Affirmative Action/Equal Opportunity Employer consistent with applicable federal and state law. All qualified applicants are encouraged to apply.*

BIOCHEMISTRY FACULTY POSITION

The Department of Chemistry at the University of Nevada, Las Vegas (UNLV) is accepting applications for an **ASSISTANT PROFESSOR** of Biochemistry. This full-time, 9-month, tenure-track position will begin August 1, 2011. The biochemistry-related degree programs within the UNLV Department of Chemistry include Biochemistry B.S., Biochemistry M.S., and Chemistry Ph.D. (includes a biochemistry track). Successful applicants will be expected to develop and direct an externally funded research program, teach at the undergraduate and graduate levels, and mentor students. A Ph.D. in Biochemistry or a related field is required; postdoctoral experience is strongly preferred. This position will include the opportunity to participate in an NIH-funded INBRE program; therefore, research interests in a biomedically related area are preferred. However, all areas of biochemistry will be considered. Salary is competitive with those at similarly situated institutions. Position is contingent upon funding. Further information about the UNLV Chemistry programs can be found at **website: <http://sciences.unlv.edu/Chemistry/>**. Submit a letter of interest, curriculum vitae, a document to include both a research plan or outline (three to four pages) and a statement of teaching philosophy including topics suggested for incorporation into the graduate curriculum (one to two pages). Materials should be addressed to **Dr. Ronald Gary, Search Committee Chair**, and are to be submitted via online application at **website: <https://hrsearch.unlv.edu>**. In addition, applicants should provide the names, addresses, telephone numbers, and e-mail addresses of three individuals who will provide letters of reference. The review of materials will begin immediately, and will continue until the position is filled. For assistance with UNLV's online applicant portal, contact **Jen Martens** by **telephone: 702-895-2894** or **e-mail: hrsearch@unlv.edu**. *UNLV is an Equal Opportunity/Affirmative Action Educator and Employer committed to excellence through diversity.*

FELLOWSHIPS



HARVARD UNIVERSITY BULLARD FELLOWSHIPS IN FOREST RESEARCH

Each year Harvard University awards a limited number of Bullard Fellowships to individuals in biological, social, physical and political sciences to promote advanced study, research or integration of subjects pertaining to forested ecosystems. The fellowships, which include stipends up to \$40,000, are intended to provide individuals in mid-career with an opportunity to utilize the resources and to interact with personnel in any department within Harvard University in order to develop their own scientific and professional growth. In recent years Bullard Fellows have been associated with the Harvard Forest, Department of Organismic and Evolutionary Biology and the J. F. Kennedy School of Government and have worked in areas of ecology, forest management, policy and conservation. Fellowships are available for periods ranging from six months to one year after September 1, 2011. Applications from international scientists, women and minorities are encouraged. Fellowships are not intended for graduate students or recent postdoctoral candidates.

Information and application instructions are available on the Harvard Forest web site (<http://harvardforest.fas.harvard.edu>). Annual deadline for applications is **February 1, 2011**.

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POSITIONS OPEN



HARVARD UNIVERSITY FACULTY POSITION IN THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

The School of Engineering and Applied Sciences (SEAS) and the Center for Brain Science (CBS) at Harvard University are seeking a tenure-track Assistant Professor in the area of theoretical and computational neuroscience. We are especially interested in individuals with a broad background and interest in modern applied mathematics.

As a member of SEAS and CBS, the successful candidate will join one of the areas within SEAS such as applied mathematics, applied physics, bioengineering, computer science, or electrical engineering, and have opportunities to interact with faculty members from other areas within SEAS as well as other departments in the Faculty for Arts and Sciences, such as physics and molecular and cellular biology. The aim of CBS is to discover the structures and functions of brain circuits via interactions across disciplinary boundaries. Faculty from several academic departments are housed in common research space, very close to that of the rest of SEAS.

Candidates should have demonstrated excellence in both research and teaching. Teaching opportunities will include offerings at both undergraduate and graduate levels. Applications from, or nominations of, women and minority candidates are encouraged.

To apply, please send a cover letter, curriculum vitae, and statements of research and teaching interests, and arrange for submission of 3 letters of recommendation. Application materials should be e-mailed to: cbs@fas.harvard.edu. Review of applications and nominations will begin **15 November 2010**. Further information about the School of Engineering and Applied Sciences and the Center for Brain Science is available at their respective websites: <http://www.seas.harvard.edu/> and <http://cbs.fas.harvard.edu/>.

*Harvard University is an
Affirmative Action/Equal Opportunity Employer.*

CONFERENCE

The 8th Okazaki Biology Conference "Speciation and Adaptation II – Environment and Epigenetics - "

Okazaki, Japan, March 25-29, 2011

The Okazaki Biology Conferences (OBC) hosted by the National Institute for Basic Biology (NIBB), one of Japan's leaders in promoting international cooperation in the field of biology, are international conferences dedicated to providing opportunities to create new international networks of scientists as well as to facilitate the development of future areas of research in the biological sciences. The conferences are held in relaxing intimate settings that allow participants to get the most out of the time spent.

The theme of the 8th OBC will be "Speciation and Adaptation II – Environment and Epigenetics". "Speciation and adaptation" is a fundamental research area of evolutionary biology, continuously challenged by researchers from various scientific disciplines. In the Conference, we will explore a new direction of evolutionary studies by integrating theoretical and experimental genetics, environmental genomics, and epigenetics, using variety of organisms. Speakers include Greg Gibson, Jenniffer Graves, Marilyn Renfree, Chung-I Wu, Luca Comai, Andrew Leaky, Tetsuji Kakutani and Organized by Michael Purugganan (NYU), Fumitoshi Ishino (TMDU), Mitsuyasu Hasebe (NIBB), and Aya Takahashi (NIG).

NIBB is calling for speakers to contribute to this conference. Traveling and lodging expenses will be provided for those selected.

For more information please see the link to the 8th OBC homepage below.

Registration Process and Deadline: Please use the link below to download the application form and e-mail the completed form to obc8@nibb.ac.jp by November 11, 2010.

National Institute for Basic Biology
TEL: +81 (0)564-55-7596
E-mail: obc8@nibb.ac.jp
URL: <http://www.nibb.ac.jp/obc/8th/>



POSITIONS OPEN

ASSISTANT PROFESSOR OF BIOLOGY Microbiology Biology

Hofstra University anticipates an opening for a full-time, tenure-track, assistant professor in the Department of Biology. The successful candidate must have experience teaching microbiology (both to biology majors and pre-allied health students) and at least one other of the following subject areas: molecular biology, immunology, or biotechnology at the undergraduate level. The successful candidate will be expected to teach courses at a variety of levels, i.e. non-majors, undergraduate majors, and Master's. Faculty in Biology must maintain an active, productive and externally funded research program accessible to both undergraduate and master's research students. The specific areas of research are open. Candidates must have a Ph.D. in a relevant discipline, experience in course development and design, and demonstrated teaching ability. Experience with innovative teaching techniques is desirable. Postdoctoral experience is required. Interested individuals should submit: (1) curriculum vitae (indicating specific teaching experience); (2) statement of research interests; (3) statement of teaching experience, interests, and philosophy; and (4) have letters from three references sent to: **Faculty Search, Department of Biology, Hofstra University, Hempstead, NY 11549-1140**, or electronically send application documents (in PDF format) to **e-mail: robert.w.seagull@hofstra.edu**.

The Department of Biology is a medium-sized, diverse, and growing department. Additional information about the department, faculty, facilities, etc. is posted on the **website: http://hofstra.edu/biology**.

Application review will begin November 15, 2010 with a starting date in September 2011.

Hofstra University is an Equal Opportunity Employer, committed to fostering diversity in its faculty, administrative staff and student body, and encourages applications from the entire spectrum of a diverse community.

POSTDOCTORAL FELLOW in BIOENERGY University of Rochester, Rochester, New York

Postdoctoral positions are available for studying gene regulation of biomass degradation enzymes (cellulases) in the thermophilic bacterium *Clostridium thermocellum* for bioethanol and biohydrogen productions. Background in molecular biology, microbiology, and biochemistry is desirable. Expertise in transcription regulation and/or genomic and proteomic approaches is beneficial. Candidates with a Doctoral degree in life science or bioengineering are encouraged to apply. Please submit electronically the curriculum vitae with information of three references to **Professor David Wu** at **e-mail: davidwu@che.rochester.edu**.

POSTDOCTORAL RESEARCHERS - Seeking two highly motivated individuals to conduct NIH-funded research on structural virology focusing on genome packaging of DNA viruses by means of X-ray crystallography and electron cryo-microscopy in **Dr. Liang Tang's** laboratory at the Department of Molecular Biosciences, University of Kansas. Requires a Ph.D. in biochemistry or a related field with a strong background in protein crystallography. For more information and to apply go to **website: https://jobs.ku.edu** and search for the job number **00067319**. Review of applications begins October 21, 2010, and will continue until the positions are filled. *Equal Opportunity/Affirmative Action Employer.*

ASSISTANT or ASSOCIATE PROFESSOR Eukaryotic Cell/Molecular Biologist

The Department of Biological Sciences at California State University, Long Beach invites applications for a tenure-track Assistant or Associate Professor starting August 22, 2011. We seek broadly trained applicants who address fundamental research questions in eukaryotic cellular processes. For further information, please see the position description at **website: http://www.csulb.edu/divisions/aa/personnel/jobs/cnsm/**.

California State University, Long Beach is an Equal Opportunity Employer.

POSITIONS OPEN

UNIVERSITY OF CALIFORNIA, IRVINE Tenured or Tenure-track Faculty Position in Systems Biology

The University of California, Irvine (UCI) is renewing its recruiting initiative in Systems Biology.

Two positions are available this year, for which candidates will be considered from all areas of Systems Biology, including biological networks, regulatory dynamics and control, spatial dynamics and morphogenesis, synthetic biology, and mathematical and computational biology. Applications are being solicited at the **ASSISTANT PROFESSOR** level, and appointment can be made in any of several departments, including Developmental and Cell Biology, Molecular Biology and Biochemistry, Ecology and Evolutionary Biology, Biomedical Engineering, Mathematics, Physics and Astronomy, Computer Science, and Statistics. Applications at the Associate and Full Professor level will also be considered, with appointment being subject to the availability of funds.

The successful applicant is expected to conduct a strong research program and to contribute to the teaching of undergraduate and graduate students. Systems Biology research and training at UCI is fostered by several interdisciplinary research units, an NIGMS National Center for Systems Biology, and Ph.D. training programs in Bioinformatics, and Mathematical and Computational Biology (for more information, see **website: http://ccbs.bio.uci.edu**). Applicants should submit a letter of application, curriculum vitae, bibliography, three letters of reference, and statements of research and teaching interests using the online recruitment system (see instructions at **websites: http://ccbs.bio.uci.edu** or **https://recruit.ap.uci.edu**, under "Institutes and Centers"). To receive full consideration, material should be received by December 10, 2010.

The University of California, Irvine is an Equal Opportunity Employer committed to excellence through diversity, and strongly encourages applications from all qualified applicants, including women and minorities. UCI is responsive to the needs of dual career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF ADVANCE Award for gender equity.

POSITIONS OPEN

FACULTY POSITION in Plant Genetics

The Department of Genetics at the University of Georgia (UGA) invites applications for a tenure-track faculty position in any area of plant genetics at the **ASSISTANT PROFESSOR** level. The candidate will be expected to maintain a rigorous, externally funded research program and to contribute to undergraduate and graduate teaching. For information about the breadth of the department, see **website: http://www.genetics.uga.edu** and plant biology in general at UGA, see **website: http://plantcenter.uga.edu/index.php**.

Applications should be sent electronically as a single PDF file (filename format: yourlastname.pdf) that includes a cover letter, curriculum vitae, and brief statements of research and teaching interests to **e-mail: genetics@uga.edu**. Three letters of recommendation should also be sent, either as a PDF to the above e-mail address (filename format: applicantlastname_refereclastname.pdf), or in hard copy, to: **Plant Genetics Search Committee, Department of Genetics, Davison Life Sciences Building, University of Georgia, Athens, GA 30602-7223**. The committee will begin reviewing applications on December 1, 2010, until the position is filled.

The Franklin College of Arts and Sciences, its many units, and the University of Georgia are committed to increasing the diversity of its faculty and students, and sustaining a work and learning environment that is inclusive. Women, minorities and people with disabilities are encouraged to apply. The University is an Equal Employment Opportunity/Affirmative Action Institution.

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